FUNGAL BIOLOGICAL CONTROL OF *HIERACIUM*

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Abstract

Hieracium species are a severe weed problem in the high country native tussock grasslands of New Zealand. This thesis reports on the potential for fungal biological control of *Hieracium*, in particular with a rust pathogen, *Puccinia hieracii* var. *piloselloidarum*.

Isolates of *Hieracium* rust were collected from throughout Northern, Central and Southern Europe, and the British Isles. One thousand four hundred and twenty four isolates were screened on New Zealand *Hieracium pilosella* to identify the most infective strains for potential use as biological control agents. The rust isolates most pathogenic to New Zealand *H. pilosella*, were from the south of Ireland. They had a shorter latent period and higher infectivity compared to other isolates. One isolate infected representatives of all New Zealand *H. pilosella* sites as well as *H. praealtum* and *H.* x *stoloniflorum*.

Hieracium rust was common throughout Europe with large seasonal fluctuations. Most dissemination, infection and effect was seen in a main peak in spring and a secondary peak in autumn. The rust could survive through winter conditions within host tissue allowing rapid re-establishment of symptoms during occasional periods of suitable milder weather and, eventually, with the onset of spring. In an intensive field study of one Edinburgh area, the level of rust infection on patches of *H. pilosella* was found to be affected by several site factors, particularly the density of patches.

The infection process of *Hieracium* rust was studied. Spore germination was fastest in the dark and occurred over a wide range of temperatures. Inoculations of hosts was either on to detached leaves kept on water agar or on to whole rosettes. Infection of detached leaves was generally higher than on whole rosettes and may allow a wider host range of subgenus *Pilosella* taxa. Infection rarely occurred on all inoculated plants. This was attributed in part to the effect of host condition. A genetic resistance component of the non-susceptibility remains possible although one rust isolate was able to infect representatives of all identified genotypes of New Zealand *H. pilosella*.

The variation present in New Zealand *Hieracium* species was investigated by chromosome analysis and isozyme electrophoresis. *H. pilosella* from 34 collections throughout New Zealand were predominantly pentaploid with a hexaploid found in just one population; the pentaploids included variants, according to electrophoresis results and morphological characters.

Hieracium rust showed potential as a biological control agent. The rust significantly affected the growth of *H. pilosella* and displayed strict host-specificity, with no hosts outside the subgenus *Pilosella*.

Powdery mildew, *Erysiphe cichoracearum*, is common and very pathogenic on *Hieracium* spp. throughout Europe. However, *Hieracium* powdery mildew grew on two New Zealand endemic species, *Embergeria grandifolia* and *Kirkianella novae-zelandiae*. Several other *Hieracium* pathogens were noted but their potential for biological control requires further investigation.

CHAPTER I. INTRODUCTION

I.1 GENERAL

Species of *Hieracium* L. were accidentally introduced to New Zealand from their native range of Europe and have become a weed problem in much of the South Island high country. The present scale of this weed problem and the lack of control methods in New Zealand prompted investigations into the possibilities of biological control programmes. This thesis concerns the potential for fungal biological control of *Hieracium* weed species in New Zealand. Most work focussed on the rust fungus, *Puccinia hieracii* (Röhl) Mart. var. *piloselloidarum* (Probst) Jørstad which infects members of *Hieracium* subgenus *Pilosella* Hill.

An introduction is presented below, reviewing the *Hieracium* problem (Section I.1.1), biological control of weeds (Section I.2), the *Hieracium* genus (Section I.3) and pathogens of *Hieracium*, particularly rust (Section I.4). Previous work on fungal biological control of *Hieracium* is described (Section I.5) and the consequent aims of the thesis are outlined (Section I.6).

I.1.1 *HIERACIUM* WEED PROBLEM

I.1.1.a *Hieracium* taxa present in New Zealand

There are nine species of *Hieracium* naturalised in New Zealand (Garnock-Jones in Webb *et al.*, 1988). Four species are in subgenus *Pilosella* and five species are in subgenus *Hieracium*. There is also an interspecific hybrid *H.* x *stoloniflorum*, with ancestral parentage of subgenus *Pilosella* species *H. aurantiacum* and *H. pilosella*. The taxa and their relative weed significance in New Zealand are listed in Table 1.1.

Hieracium species are considered to be a serious problem throughout large areas of the South Island high country. *H. pilosella* is by far the most serious weed species of the genus in New Zealand (Scott, 1984). Hunter (1991) reported on the distribution of *Hieracium* species and their perceived weed status; he described around 500 000

hectares of land as *Hieracium* (mostly *H. pilosella*) dominant. The Waitaki Basin (Canterbury) and the upper Awatere Valley and Clarence tributaries (Marlborough) are some of the most seriously affected areas. *H. caespitosum* (particularly in Marlborough and Otago) and *H. praealtum* (particularly in South Canterbury and Otago) are also reported as major weed problems. Of the subgenus *Hieracium*, *H. lepidulum* has been recorded as a dominant weed problem in the Manorburn area (Central Otago) and Saxton Pass (Marlborough).

In the North Island, where *H. pilosella* is already common, the species may pose a threat to the conservation value of remnant tussock grassland communities (Hunter, 1991).

Table 1.1. Taxa of *Hieracium* present in New Zealand, and their weed status.

Subgenus	Binomial	Weed Status	Main Areas Affected[a]	First Record in NZ
Pilosella	*H. pilosella* L.	Immense	M, C, O	1878
Pilosella	*H. praealtum* Vill. ex Gochnat	Very Large	C, O	1924
Pilosella	*H. caespitosum* Dumort.	Large	M, C, O	1940
Hieracium	*H. lepidulum* (Stenström) Omang.	Large	C, O	1946
Pilosella	*H.* x *stoloniflorum* Waldst. & Kit.	Medium	C	1920
Pilosella	*H. aurantiacum* L.	Medium	C	1911
Hieracium	*H. murorum* L.	Small	C	1940
Hieracium	*H. argillaceum* group	Insignificant		1940
Hieracium	*H. sabaudum* L.	Insignificant		1904
Hieracium	*H. pollichiae* Schultz-Bip.	Insignificant		1988

The first records are mostly from Garnock-Jones (in Webb *et. al.*, 1988) but the first record of *H.* x *stoloniflorum* is an herbarium specimen at Lincoln CHR 1308, collected by H.H. Allan, originally recorded as *H. aurantiacum* but correctly identified by P.J. Garnock-Jones.

[a] Areas Affected : M = Marlborough, C = Canterbury, O = Otago.

Hieracium subgenus *Pilosella* has been divided into two groups. *H. pilosella* is the sole representative of the *Acaulia* group in New Zealand while the *Cauligera* group has three representatives, *H. aurantiacum*, *H. caespitosum* and *H. praealtum*. European representatives of these three species have tested negative for umbelliferone (a phenolic chemical of potential taxonomic value in this subgenus) while members of the *Acaulia* group have tested positive (Bate-Smith *et al.*, 1968). *H.* x *stoloniflorum* is a hybrid between the two groups and European *H.* x *stoloniflorum* has tested positive

for umbelliferone (Bate-Smith *et al.*, loc. cit.). Makepeace *et al.* (1985) reported that umbelliferone was present in leaves of New Zealand *H. pilosella* but not in leaves of *H. praealtum*.

Although first recorded in New Zealand before 1880 (Murphy, 1878), it was not until the latter half of the 20th century that *H. pilosella* was common in the New Zealand high country. Allan (1924) reported on the presence of *Hieracium* in Canterbury; *H. pilosella* was well established near Hinds River, but was thought to be able to be eradicated if serious control measures were adopted, and *H. praealtum* was so well established, especially at Mount Peel and Orari Gorge, that the chance of eradication was remote. Healy (1969, p.315) noted that *H. lepidulum* (recorded as *H. lachenalli*), *H. pilosella*, and *H. praealtum* had increased since the 1940's and reported extensive colonies of *H. pilosella* in the Burkes Pass locality. In the mid 1940's, *H. lepidulum* was noted as established in pasture of the Avoca Valley, Canterbury (Healy, 1946). Healy (1957) recorded *H. caespitosum* as being thoroughly established on shady faces of the Wairau Valley, Marlborough.

Connor (1992, p.247) described *H. pilosella* and *H. praealtum* as having been "widespread and well established" in the 1960's. Generally abundance of *Hieracium* spp. was low, though at a few of Connor's relevés, *H. pilosella* accounted for 25 to 50% of the area and at two relevés, *H. praealtum* accounted for a similar percentage of area. Concern about the weed threat of *H. lepidulum* was reported in the 1970's by Dunbar (1977). *Hieracium* has only recently achieved great weed significance after a long period of ingression and establishment in the high country. Scott (1984) suggested that the dramatic increase of *H. pilosella* and *H. praealtum* in the high country may have been associated with open vegetation, a series of warm wet summers and reduced rabbit grazing at the end of the 1950's rabbit era.

The weed problem of *Hieracium* spp. is the displacement and suppression of vegetation after they have established in an area. The economic impact of *Hieracium* infestations in the New Zealand high country was reviewed by Grundy (1989). Fine wool production has been the main agricultural industry affected. The agricultural

concern for *Hieracium* is limitation of stock feed (Scott, 1993). The displacement of vegetation has also compromised nature conservation values of the high country.

The loss of native tussocks, and their shelter, is perhaps more important than the actual percentage of ground covered by vegetation. The microclimate provided by fescue tussock shelter is probably beneficial for seedling establishment (Scott, 1961; 1975b). Gradwell (1954) found that shading from tussocks reduced rapid freezing and thawing of the soil surface therefore limiting "needle" ice and frost heaving, a major cause of seedling mortality in the New Zealand high country (Gradwell, 1954; Simpson and Moore, 1955).

I.1.1.b *Hieracium* taxa naturalised in other countries

Hieracium species of subgenus *Pilosella* are recognised as weed problems in North America. These include *H. pratense* Tausch (= *H. caespitosum* Dumort., according to Sell and West, 1976; Voss and Böhlke, 1978), *H. floribundum*, *H. aurantiacum* and *H. pilosella*. Myers (1950) described *H. pratense* and *H. floribundum* as two of the most troublesome and fast spreading weeds in West Virginia, USA.; the species spread across much of the state in comparatively few years, covering old field meadows. The United States Department of Agriculture has initiated a biological control programme for naturalised *Hieracium* species in the USA. One of the targets of the programme is *H. pratense* (L. Fornasari, pers.comm.).

Naturalised *H. aurantiacum* is also recorded as a weed in Japan (Suzuki and Narayama, 1977). *H. pilosella* is present in southern Chile and appears to be increasing in abundance (N. Covacevich, pers. comm.). The species has been present in the south of South America since at least 1897 (Macloskie, 1903-1906). Groves (1986), following experience of the species in New Zealand, recognised the risk of a potential *Hieracium* invasion in certain parts of south eastern Australia. *H. pilosella* may be locally naturalised in Tasmania (R. Groves, pers.comm.).

I.1.2 POTENTIAL IMPACT OF *HIERACIUM* CONTROL

I.1.2.a. Impact of *Hieracium* Control on Economic Activities

Grundy (1989) gave a conservative estimate of $1.1 to $4.4 million annual reduction in value of high country agricultural production caused by *Hieracium*. Although Grundy did not attempt to estimate the cost of the *Hieracium* weed problem to conservation, he described this as significant. The effect of successful biological control would alleviate a proportion of these costs.

Grundy (1989) considered that *Hieracium* had negligible benefits in comparison to the above costs. Economic returns from *Hieracium* are limited. There is a small annual supply of 10 kg of *H. pilosella* seed to Europe for the herbal industry (Grundy, 1989). There are some commercial nursery sales of orange hawkweed (*H. aurantiacum* or *H.* x *stoloniflorum*).

Hieracium does provide stock feed but its availability (especially for *H. pilosella*) is much lower than the species it displaces.

Grundy (1989) noted that *Hieracium* was possibly locally important for honey production in some areas. Moar (1985) reported that no more than 1% of the pollen count of honey from *Hieracium* areas was of the *Taraxacum* type (which includes *Hieracium*). In a study of the nutrient composition of pollen collected by honey-bees in Otago, Day *et al.* (1990) found that *Hieracium* (presumably mostly *H. pilosella*) pollen was low in every mineral analysed and had below the normal honey-bee requirement of isoleucine. *Hieracium* appeared to have the lowest nutritive value of all the pollens tested in terms of amino acids, and Day *et al.* (1990) noted that this may contribute to the protein deficiency observed in some bee colonies.

I.1.2.b. Impact of *Hieracium* Control on Conservation

Control of *Hieracium* would relieve the suppression of native and agricultural species in the high country. The potential reduction of soil cover from *Hieracium* control requires consideration. When *H. pilosella* is controlled with herbicide, dead patches can remain intact for about two years (D. Scott pers. comm.). In most areas of the New Zealand high country, this would allow time for other plant species to become established following control.

I.2 BIOLOGICAL CONTROL OF WEEDS

I.2.1 GENERAL

Biological control of weeds involves using another organism, termed a biological control agent (BCA), to control plant populations. Although the majority of weed BCAs employed have been entomological, interest has increased in microbial, and particularly fungal, agents. A number of weed biological control programmes have spectacularly reduced weed populations to levels below economic thresholds, but the majority have not been successful (Julien *et al.*, 1983).

There are two main approaches to fungal biological control, classical or inundative (or mycoherbicidal). Classical biological control involves the introduction of a BCA to a new area where the host is a problem weed and the BCA spreads and self perpetuates. The ultimate intention is to reduce or eliminate the weed problem by reducing the vigour or reproductive capacity of the weed.

Inundative biological control is a more recent approach which involves the application of a large dose of a pathogen. The pathogen is often already present on the weed populations at low levels. The aim is for increased application of the propagated pathogen to eradicate, or at least severely reduce, the vigour of the target weed.

An augmentative method incorporates aspects of classical and inundative approaches, by increasing the level of a pathogen already present on weed populations.

Augmentative biological control can involve provision of extra inoculum of the pathogen, or changing the environment (for example, with strategic irrigation) to favour the pathogen.

This thesis is concerned almost entirely with the classical biological control approach.

I.2.2 CLASSICAL BIOLOGICAL CONTROL OF WEEDS

I.2.2.a Introduction

Classical biological control has been practised for over a century. Traditionally, most BCAs employed in weed control have been insects. Typically the target weed has been inadvertently introduced from its native habitat. The BCA is collected from its native distribution where it has been observed to have some effect on the weed species. Successful biological control would result in a significant reduction of weed populations.

It is undesirable for economic or important conservation species to be detrimentally affected by a BCA and host specificity is a principle component of an agent's suitability for introduction. The ability of a host specific pathogen to control a weed population without a direct negative effect on surrounding vegetation is one of the strong advantages of classical biological control programmes. A significant economic advantage of classical biological control is the self spread and long term presence of control without the need for intervention. Thus classical biological control has often been employed for weeds affecting large tracts of land where economic return of agriculture or other activities cannot afford other control methods such as herbicide application or tillage. The usefulness of this classical BCA in extensive rangeland was demonstrated by the clearing of millions of hectares infested with *Opuntia* B.& R. species (Dodd, 1959).

The financial cost of a fungal biological control programme is typically in the initial research and introduction of a pathogen (Auld, 1991). There is a risk that biological

control will be unsuccessful, therefore biological control programmes usually include an early assessment of the potential for a BCA to control the target weed. In the case of pathogens there is often a comparison of strains to find the most effective.

New Zealand was one of the first countries to investigate the potential of fungal biological control. Cockayne (1914; 1915; 1916) reported that *Puccinia punctiformis* (Strauss) Roehling rust of Californian thistle (*Cirsium arvense* (L.) Scop.) was widespread in New Zealand and locally severe. Cockayne suggested a procedure of enhancing the effect of the rust through artificial inoculations. The work of Cockayne was an example of augmentative control rather than purely classical biological control. Cunningham (1927) reviewed the potential for fungal biological control of weeds in New Zealand, and saw little value in this method given the limited knowledge and techniques available at the time. He reported that even when following Cockayne's method of artificial inoculations of *Cirsium arvense* with the rust pathogen, only 50% of plants were infected. Overseas and more recently, attention has focussed on inducing teliospore production of *P. punctiformis* to sufficient potential for mycoherbicidal control of *C. arvense* (Völker and Boyle, 1994).

The first example of an attempt at purely classical biological control reported in New Zealand is described by Cunningham (1927), who sent a worldwide request for isolates of fungal pathogens of blackberry (*Rubus fruticosus* agg.). Among the isolates received were two rust fungi, *Phragmidium violaceum* (Schulz) Wint. (from England) and *Gymnoconia peckiana* (Howe) Trotter (from the USA). Inoculations of the isolates onto New Zealand plants were, however, unsuccessful. More recently, a planned introduction of *P. violaceum* rust into Chile resulted in successful control of blackberry weed species (Oehrens & González, 1974).

Fungal classical BCAs have commonly been rust fungi (Hasan and Ayres, 1990). Some of the positive characteristics of rusts as BCAs include their very limited host range and their generally good dissemination of spores. There are examples of rusts that can kill their host, e.g., *Puccinia chondrillina* Bub. & Syd. on *Chondrilla juncea*

L. (Hasan and Wapshere, 1973), *P. punctiformis* on *Cirsium arvense* (Forsyth & Watson, 1985) and *Uromyces heliotropii* on *Heliotropium europaeum* L. (Hasan and Aracil, 1991) and many examples of rusts that reduce host growth and reproductive capacity. Wager (1947) attributed extensive reductions of bramble (*Rubus fruticosus bergii*) populations in Natal, South Africa, to the rust *Kuehneola uredinis* (Link) Arth.. Rust fungi have been proposed for biological control of a variety of plants which pose problems as weeds in rangeland agricultural areas (Alber *et al.*, 1986; Bruckart, 1989).

A good example of classical biological control of weeds with fungi is that of *Chondrilla juncea*. A rust pathogen, *P. chondrillina*, was screened in Europe to find a highly pathogenic strain for the Australian populations of *C. juncea*. Introduction of one strain to Australia in 1971 steadily reduced the economic effect of the weed (Burdon *et al.*, 1981). *Cystiphora schmidti* (skeleton weed gall midge) and *Aceria chondrillae* (skeleton weed gall mite) were also introduced for biological control of *C. juncea* but were not found to be as damaging as the rust fungus (Delfosse *et al.*, 1985). *P. chondrillina* was later introduced into California where it contributed to 56 to 87% reductions in rosette density of some *C. juncea* populations (Supkoff *et al.*, 1988).

The *P. chondrillina* rust strain released into Australia only infected the then main problem form ('A') of the weed (Burdon *et al.*, 1981). In Australia, forms 'B' and 'C' of *C. juncea* were not infected by the introduced rust strain and increased their weed significance (Burdon *et al.*, 1984). Searches for strains of *P. chondrillina* pathogenic to the 'B' and 'C' forms have concentrated on areas of Turkey and Greece. Two strains have been introduced for form B and only a few weakly pathogenic strains have been found to infect form 'C' (Hasan, 1985).

Biological control of *C. juncea* with *P. chondrillina* was more effective in the higher rainfall New South Wales target areas than in the drier and light soil areas of South Australia and Victoria. Even in these latter areas, wheat harvest losses attributed to *C. juncea* were alleviated by between 66% and 86% (Cullen *et al.*, 1973):.

The present investigation of biological control of *Hieracium* with a *Puccinia* rust was modelled on the rust biological control of *C. juncea*. *C. juncea* is also a member of the Lactuceae and, similar to *Hieracium*, the species is perennial, apomictic and forms herbaceous rosettes. *P. chondrillina*, as with *P. hieracii* var. *piloselloidarum*, has a specific host on which all stages of the rust's life cycle are possible.

I.2.2.b Predictions of success

A method for predicting the success of weed biological control by an insect was proposed by Harris (1973). The general concepts employed in this method are also relevant to the assessment of a pathogen's suitability for weed biological control. The method is based on aspects of the agent's biology such as generation time and the interaction with the environment. The assessment includes the damage that the agent inflicts on target weeds. Further aspects to consider in predicting the success of fungal biological control are the agent's transmissibility and the proportion of hosts that are susceptible.

Field observation and supplementary experimentation can indicate the ability of the pathogen to spread, as well as the adaptability to climatic situations similar to those in the new environment proposed for agent release. The effect of the pathogen on host growth can be quantitatively measured, either in glasshouse conditions or in the field with local host populations which may differ genetically from populations in the proposed area of introduction. While such studies may be indicative, the interaction of pathogen, host and environment is so complex, that the only definitive test is to actually attempt biological control. The measurement may also ignore the indirect effect of a BCA on the target host, such as increased susceptibility to drought, cold, other organisms and plant competition.

I.3 THE GENUS *HIERACIUM*

I.3.1 TAXONOMY

I.3.1.a Asteraceae

The Asteraceae family is large and comprises two subfamilies. Subfamily Cichorioideae contains five tribes including the Lactuceae, to which the genus *Hieracium* belongs. There are six native New Zealand species in the Cichorioideae, all belonging to the Lactuceae. In New Zealand there are at least 64 adventive species of the Cichorioideae. The main crop and pasture species in this group include globe artichoke (*Cynara scolymus* L.), and in the Lactuceae specifically are, lettuce (*Lactuca sativa*), chicory (*Cichorium intybus* L.), endive (*Cichorium endivia* L.), and puwha (*Sonchus oleraceus* L.). Several species have value as ornamental plants. The subfamily includes many weed species such as *Cirsium* Miller species (thistles).

The other subfamily, Asteroideae, contains eight tribes and includes the crop plants, sunflower (*Helianthus annuus* L.) and Jerusalem artichoke (*Helianthus tuberosus* L.), and many ornamental daisy species. This subfamily includes 264 native species.

I.3.1.b *Hieracium*

Hieracium consists of two distinct subgenera, namely *Hieracium* (Euhieracia) and *Pilosella*. Species of both subgenera share the common name of hawkweed. Whilst both subgenera have apomictic taxa, several cytological races of some species can reproduce sexually, and interspecific hybridisation is possible. Sell (1987) argued that members of the mainly American subgenera *Stenotheca* Fries and *Mandonia* Arvet-Touvet were better assigned to either existing or new genera. Thus the European representative of subgenus *Stenotheca*, *H. staticifolia* All. is treated in the genus *Tolpis* Adans. by Sell and West (1976).

Sell and West (1974) noted that intermediates between most described *Hieracium* taxa are present within the subgenera, but that there were no intermediates between taxa of different subgenera. It was for that reason, as well as the existence of distinct

morphology, that Sell and West (1968, 1974) advocated that *Hieracium* and *Pilosella* were best considered as separate genera. The genus *Pilosella* Hill was named by Hill (1756) and later defined by C.H. & F.W. Schulz. However, Sell and West (1976, p. 358) in the Flora Europaea, classified *Pilosella* as a subgenus because the Editorial Committee, "... having reviewed the opinions of the Regional Advisers, decided that for the purposes of this Flora they would be best united in order to maintain nomenclatural continuity with the work of Zahn and the majority of European Floras." *Pilosella* was treated as a separate genus by Sell and West in the Flora of Turkey (see Sell and West, 1974) and more recently by Stace (1991) in the New British Flora. Stace (loc. cit.), however, described *Pilosella* as doubtfully distinct from *Hieracium*.

In a review of taxonomic work on *Hieracium*, Sell (1987) outlined the morphological basis for separating *Pilosella* at the genus level. Sell described the genus *Hieracium* and the genus *Pilosella* as particularly distinct in achene characteristics along with general facies. *Pilosella* achenes are less than 2.5 mm in length and have pappus hairs in one series whilst *Hieracium* achenes are 3 to 5 mm long and have two series of hairs. Achene characteristics are an important component in the taxonomy of the Lactuceae.

Some New Zealand reports have employed binomials that treat *Pilosella* as a genus (for example Scott, 1984). In the Naturalised Flora of New Zealand however, *Pilosella* was given subgenus status and the arguments for separate genus status were described as not all convincing (Garnock-Jones in Webb *et al.*, 1988). This thesis follows the Garnock-Jones' (loc. cit.) treatment of the subgenera.

From a point of view of fungal biological control, the distinction between the two subgenera is significant. The two taxa are susceptible to morphologically distinct varieties of the rust pathogen, *Puccinia hieracii*.

I.3.1.c Subgenus *Pilosella*

The 18 species of subgenus *Pilosella* recorded by Sell (1987) are probably mostly sexually reproducing but certain cytological races of the species may be facultatively (not fully) to perhaps entirely apomictic. The apomictic nature of some cytotypes has resulted in a multitude of apparently stabilised infraspecific taxa (Turesson, 1972).

On morphological grounds, the subgenus *Pilosella* was divided into two main groups, the *Acaulia* and the *Cauligera* (Naegeli and Peter, 1885), but the groups have not been given formal ranks (Bate-Smith *et al.*, 1968). Bate-Smith *et al.* (1968) confirmed the presence of umbelliferone in all three tested members of the *Acaulia* and six taxa intermediate between the two groups. Umbelliferone was absent in all the *Cauligera* tested, and the presence or absence of the phenolic compound was therefore considered a useful taxonomic character. *H. niveum* (Muell.-Arg.) Zahn (subgenus *Pilosella*) and *Pilosella castellana* (Boiss. & Reut.) C.H. & F.W. Schultz (= *Hieracium castellanum* Boiss. & Reut.) both tested negative for umbelliferone and were treated by Bate-Smith *et al.* (1968) as species not fitting either of the two groups morphologically and chemically. *H. castellanum* Boiss. & Reuter, formerly considered part of the *Acaulia*, was thought to be better considered in a group of its own, due to a diverging form. The only intermediate between the *Acaulia* and the *Cauligera* examined and found to lack umbelliferone, was *Pilosella schultesii* (F.W. Scultz) C.H. & F.W. Schultz (=*Hieracium schultesii* F.W. Schultz). This taxon is a hybrid between *H. lactucella* Wallr. (*Cauligera*) and *H. pilosella* L. (*Acaulia*) (Sell and West, 1976).

I.3.1.d Subgenus *Hieracium* - Euhieracia

The subgenus *Euhieracium* is among the most taxonomically complicated plant genera and subgenera. Sell (1987) estimates about 10 000 species. Most representatives of the Euhieracia are apomictic and there is no evidence of these apomictic plants producing viable pollen or accepting cross pollination. The predominantly apomictic nature of the subgenus can account for the great number of species and microspecies that have been described. When variation has occurred

through mutation, somatic meiosis or sexual reproduction, then apomictic reproduction is capable of maintaining the variant genotype. Several aggregate species have been described for increased utility of *Hieracium* classifications (Sell and West, 1976).

The majority of the Euhieracia are triploid cytological races of each of the species and are apparently apomictic. The less common diploid cytotypes may reproduce sexually, and this has been confirmed for diploid *H. umbellatum* L. (Sell and West, 1974).

I.3.1.e Subspecies taxonomy

In the treatment of *Hieracium* subgenus *Pilosella*, Sell and West (1976 p. 359) employed few of the subspecies described in early European works such as Zahn (1921-1923) and Naegeli and Peter (1885). Sell and West described only those subspecies considered to be morphologically distinct and occupying a wide geographical range. Other subspecies were described as being based on insignificant characters and they usually had restricted distribution. Sell and West (1976) noted that plants intermediate in character between most subspecies exist.

The *H. pilosella* complex has been described by Omang (translated by Turesson, 1972, p. 225) as "... positively chaotic with its abundance of subspecies and varieties, their diverse inter-relationships and numerous transitional forms." Naegeli and Peter (1885) found that certain characteristics of the *H. pilosella* complex taxa remained constant after transplanting to experimental gardens. These included the length and types and distribution of hairs, size and shape of the involucre and individual bracts, peduncle length, leaf shape, leaf and flower colour and the relative lengths between internodes. Turesson (1972, p. 226) discussed the work of Naegeli and Peter (1885) and stated that the above characteristics "can with full justification be used to delimit subspecies". Turesson (1972) does however acknowledge the difficulties in *H. pilosella* subspecies identifications and considered Dahlstedt (1890) to have been too zealous in giving separate subspecific names to closely similar forms of the species.

It was also noted by Turesson that the proportion of the 2000 collections that Naegeli and Peter worked with that were *H. pilosella* sensu stricto was uncertain; therefore the extent to which these characters can be used to distinguish intraspecific variants of *H. pilosella* is not known. Sell and West (1976) employed the characters of pubescence length and distribution, involucre length and scape length to describe subspecies of *H. pilosella.*

From just 208 collections of *H. pilosella* from Sweden, Turesson (1972) reported the presence of 75 previously described subspecies. Sell and West (1976) in the Flora Europaea treatment of *H. pilosella* described only eight subspecies. Three of these are recorded in New Zealand (Garnock-Jones in Webb *et al.*, 1988) and are detailed in Table 1.2. Some herbarium specimens of New Zealand *H. pilosella* are difficult to place, either approaching descriptions of other subspecies, or appearing to be intermediates between the three recorded subspecies (Garnock-Jones in Webb *et al.*, 1988). Stace (1991) described the seven British *H. pilosella* subspecies, including the three recorded in New Zealand, as no more than varieties and as only partially or not at all geographically separated.

There are doubts about the usefulness of the subspecies due to the presence of several ploidy levels in most subspecies (see Table 1.3) indicating probable multiple origins. Just because two individuals correspond to one subspecies they may not be more closely related to each other than to members of other subspecies. The presence of subspecies in New Zealand indicates probable genetic variation in New Zealand *H. pilosella.*

Table 1.2. Involucral bract hairs of *H. pilosella* subspecies recorded in New Zealand. Hair characters for subspecies were taken from Sell and West (1976).

Subspecies	Glandular Hairs	Eglandular Hairs
micradenium Naegeli & Peter	dark or pale	none[a]
pilosella	pale (dark hair bases)	pale (dark hair bases)
trichosoma Peter	dark	dark

[a] Sell and West state that there are often pale simple eglandular hairs in the upper part of the scapes of *H. pilosella* subsp. *micradenium.*

Apart from records of the three subspecies, the presence of genetic differences within *H. pilosella* has not yet been convincingly demonstrated in New Zealand. Differences in length:breadth ratios of leaves of New Zealand *H. pilosella* reported by Makepeace (1981) remained after transplant experiments but plants were only grown in common conditions for a short term.

I.3.1.f Infraspecific hybridisation

Hybridisation between two members of the same species can confuse the infraspecific taxonomy. The existence of different ploidy levels within species indicates that autopolyplody has occurred. Even subspecies of *H. pilosella* have been recorded as having several ploidy levels (numbers of sets of chromosomes), for example subsp. *micradenium* has diploid, tetraploid and pentaploid records (Sell and West, 1976). Only apomictic reproduction has been shown in European field collections of pentaploid *H. pilosella* (Gadella, 1987). Even the apomictic pentaploid, however, produces good pollen (Gadella, 1987; Turesson, 1972) and hence the potential for hybridisation (as a pollen donor) remains. Due to the frequency of infraspecific hybridisation, caution is warranted when identifying subspecies in subgenus *Pilosella*.

I.3.1.g Interspecific hybridisation

Interspecific hybridisation is common in the *Pilosella* subgenus and has been reported in Europe and among naturalised species in North America. Sell and West (1976) state that hybrids occur between most species of subgenus *Pilosella* that occur together; they describe many of these hybrids including some with four species as putative parentage. From 18 species of subgenus *Pilosella*, Sell and West (1976) reported 111 interspecific hybrids in the subgenus; 67 were considered widespread and 44 limited in distribution. *H. pilosella* was noted as being a putative parent to 33 of the hybrids. Gadella (1992) produced hybrids, including triple hybrids (a hybrid crossed with another species) between a wide range of species of subgenus *Pilosella*. In Canada, Lepage (1967) reported several hybrids between different pairs of four

naturalised *Pilosella* species, *H. aurantiacum* L., *H. caespitosum* Dumort., *H. floribundum* Wimmer & Grab. and *H. pilosella*.

In New Zealand, the hybrid *H.* x *stoloniflorum* (= *H. pilosella* X *H. aurantiacum*) is present though it is uncertain if hybridisation occurred in this country. Garnock-Jones (in Webb *et al.*, 1988) reports herbarium specimens of New Zealand plants that may be hybrids between *H. praealtum* Vill. ex Gochnat X *H. pilosella* and *H. caespitosum* X *H. praealtum*.

Because of the alloploid nature of *H.* x *stoloniflorum*, the taxon should probably not be referred to as a species. Members of the taxon may contain varying amounts of each parent's genomes and have many different points of origin. Sell and West (1974) chose not to recognise the previously described subspecies of interspecific hybrids, suggesting that if accepted they should be termed nothomorphs to be more correct. Lepage (1967) described two nothomorphs of *H.* x *stoloniflorum* in Canada, nm. *cayouetteanum* Lepage and nm. *laurentianum* Lepage. New Zealand specimens of *H.* x *stoloniflorum* have not been assigned to nothomorphs.

I.3.2 IMPORTANCE OF PLANT TAXONOMIC WORK FOR WEED CONTROL

Weed control potential is increased by proper identification of the target weed and an assessment of the variation present. Identification allows retrieval of relevant information from the literature such as the pests and pathogens present on the species in its native range. Measuring the variation present is important to ensure that all variants of a weed are adequately controlled.

Host taxonomy can be crucial in fungal biological control with highly specific pathogens. Many obligate pathogen species have very strict host ranges, perhaps limited to a few host species. Within a pathogen species, there may be strains specific to a smaller number of host species and often races which are specific to particular genotypes of one host species. The control of *Chondrilla juncea* in Australia with the rust pathogen *Puccinia chondrillina* (Section 1.2.2.a) illustrates the importance of host

taxonomy. Because the strain of rust introduced was known to control just one form of *C. juncea*, a search for rust isolates pathogenic to the other two forms began. Isozyme electrophoresis was employed to identify forms of *C. juncea* in Europe similar to the two Australian target forms and resulted in the discovery of a large degree of *C. juncea* genetic diversity in Turkey (J. Cullen pers. comm.). From this area of high host genetic diversity, it was postulated that there may be a high diversity of rust also. Two rust strains highly virulent on the intermediate form ('B') were found in Turkey and have been released in Australia (Hasan, 1984; J. Cullen pers. comm.).

I.3.3 ECOLOGY OF *HIERACIUM*

The spread of *Hieracium* over the New Zealand high country has been a striking example of biological invasion by an alien plant. Not all introduced species have been able to invade the New Zealand countryside quite as obviously, and it is relevant to note some of the characteristics that contribute to the success of an invading species. Control measures can then aim to reduce some of these aspects of a weed's fitness.

In reviewing the characteristics of invading plant species, Groves (1986) suggested that there were several factors that predisposed a plant to becoming invasive and that these factors acted alone or in combination with each other. The attributes of members of *Hieracium* are presented below. The characteristics of *H. pilosella* and *H. praealtum* have been reviewed by Scott (1984) and *H. pilosella* characteristics have been re-reviewed by Jenkins (1992). Scott (pers. comm. 1995b) provided a comprehensive synthesis of the ecophysiological characters of *H. pilosella*. Makepeace (1980, 1985a, 1985b) reported findings on the ecology of *H. pilosella* and *H. praealtum* in New Zealand.

H. pilosella in New Zealand is most conspicuous in a rainfall range of 500 to 600 mm per annum where it was first noted as abundant, but Scott (1984) suggested that optimum environment for the species was more in the 600-800 mm rainfall zone

associated with increased soil moisture and still adequate soil fertility. Hunter (1991) reported that *H. pilosella* in the South Island high country was most abundant in a rainfall range of 600 to 1200 mm. In the North Island, humid hill country areas may be threatened (Hunter, 1991). In dry areas of the South Island high country, *H. pilosella* is often found on the shady aspects. In Britain, where the climate is overall more humid, *H. pilosella* is generally more abundant on dry banks and in exposed sites (Duffy *et al.*, 1974; Grime *et al.*, 1988).

There are two views on *Hieracium* in New Zealand. One view is that *Hieracium* species, in particular *H. pilosella*, are aggressive weeds expanding into a new niche. The other view is that *Hieracium* is an indicator of land degradation (Treskonova, 1991a, 1991b). Makepeace (1980, p.1) recognised *H. pilosella* as "an invasive species of grazed, moderate to sparsely vegetated habitats, while *H. praealtum* is an opportunist coloniser of vegetation gaps".

Some of the characteristics which help elucidate the strategy of subgenus *Pilosella* spp. are outlined below.

I.3.3.a Population biology

In the centre of patches and generally at high densities of stoloniferous *Hieracium* infestation, rosettes have reduced flower production and a lower number of stolons initiated per plant (Thomas and Dale, 1974; Makepeace, 1985a). The reduction in flowering appears to be due to a limited mineral nutrient availability (Davy and Bishop, 1984). McIntosh and Allen (1993) found no significant difference in total nitrogen levels between soil under *H. pilosella* patches and soil outside of patches, but the amount of available nitrogen (and phosphorus according to Makepeace, 1985b) per plant is likely to be much less in the centre of *H. pilosella* patches. Lloyd and Pigott (1967) increased flowering of *H. pilosella* 54 fold by fertiliser (nitrogen and phosphorus) addition three months earlier; they found that phosphorus fertiliser alone had no significant effect but nitrogen alone caused a marked increase in flowering. Because the stoloniferous *Hieracium* species are monocarpic perennials, seed set leads

to death of the flowering plants and thus fertiliser addition can accelerate population decline (Davy and Bishop, 1984).

Day length affects the physiology of *Hieracium* subgenus *Pilosella* species. *H. aurantiacum*, *H. floribundum* and *H. pratense* (=*H. caespitosum*) have all been shown to require long days for flowering (Peterson and Yeung, 1972). *H. floribundum* apparently requires five long days before producing flowers (Yeung and Peterson, 1972). *H. pilosella* flowering appears to be stimulated by long day conditions but flowering has also been noted in short day conditions. *H. pilosella* can produce generally longer stolons in long day conditions than in short days (J.P. Grime, pers.comm.). It should be noted that in *Hieracium* subgenus *Pilosella*, flowering is closely linked to stolon production, stolons usually not being produced until flowering is initiated (Thomas and Dale, 1975).

I.3.3.b Vegetative propagation and seedling establishment

The success of the stoloniferous species of *Hieracium* in the high country can be largely attributed to vegetative propagation. Seedling establishment accounts for only a small proportion of population increase of *H. pilosella* and *H. praealtum* (Makepeace, 1985a). A "window of opportunity", that is a relatively weak presence of competition and stress, is necessary for seedling establishment. In contrast, new daughter rosettes produced by stolon growth can be supported by mother rosettes (Jenkins, 1992) and therefore need not be self sufficient in photosynthesis or nutrient and moisture uptake. Vegetative spread is likely to enhance the competitive ability of *H. pilosella* and reduce the requirement for a window of opportunity. Vegetative propagation accounts for over 99% of new *H. pilosella* rosettes in sites of high weed infestation in the Mackenzie country (Makepeace, 1985a).

The strategy of *H. pilosella* can be illustrated on the Grime's triangle (Fig 1.1). The Grime's triangle contains three components of plant strategy, namely competitive ability (C), stress tolerance (S), and ability to occupy ruderal or disturbed habitats (R) (Grime *et al.*, 1988). Grime *et al.* (loc. cit.) calculated that the strategy of *H. pilosella*

was best placed between the centre (equal importance of each of the three components) of the triangle and the stress tolerance corner. The terminology for the strategy is a stress tolerator intermediate (CSR-S). This placement would have been based on a combination of the strategies of seedling and ramet propagation. The concepts of Grime *et al.* (1988) are shown in Fig 1.1 by the dotted line. Fig. 1.1 has been amended to show potential differentiation of strategy between seedling production from achenes (seed) and ramet propagation from stolons. Ramet propagation is likely to have more competitive ability than seedling production.

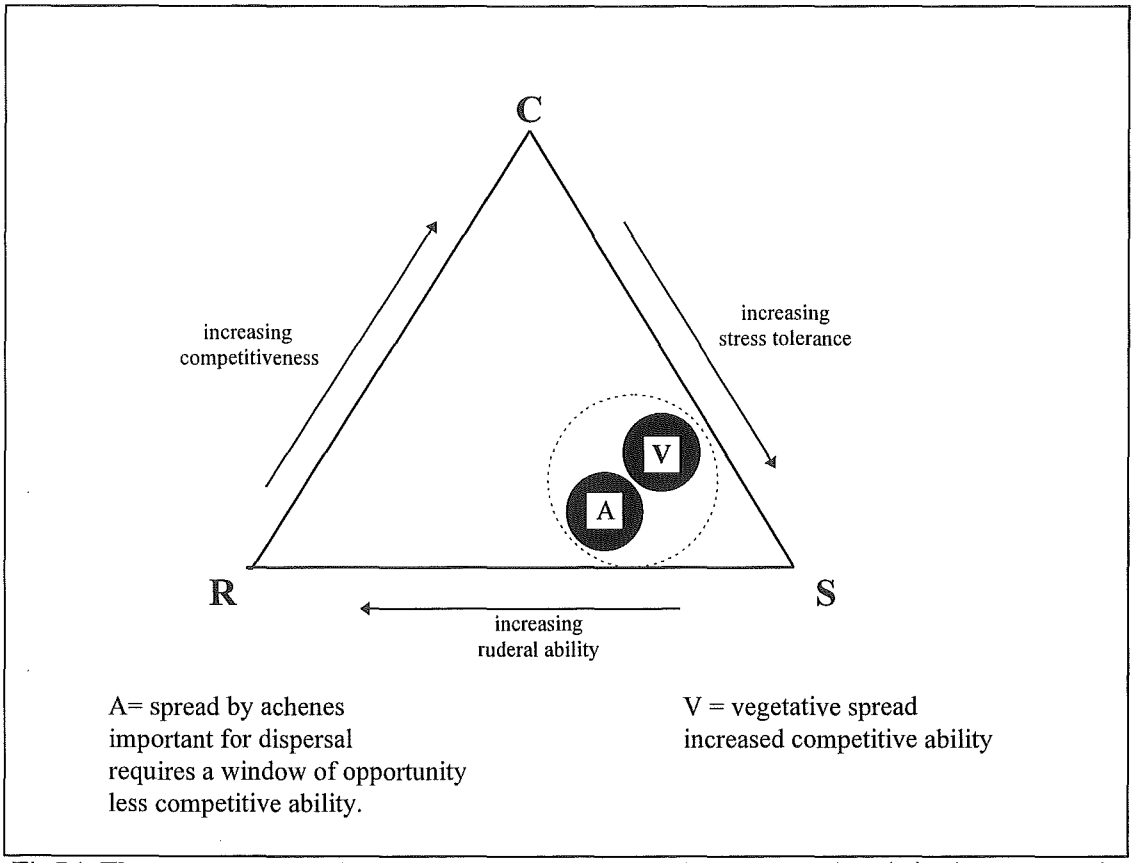


Fig I.1. The strategy of *H. pilosella* portrayed on the Grime's triangle. The relative importance of stress tolerance, ruderal ability and competitiveness of a plant's strategy is shown on the triangle by the proximity to each corner. The dotted line represents the approximate area calculated for *H. pilosella* by Grime *et al.* (1988). The separation of seedling production from vegetative propagation are suggested to emphasise the increased importance of competitive ability of the latter. The 'V' is in bold to indicate that vegetative reproduction accounts for the majority of new rosettes in established infestations.

In Britain the work of Watt (1981) pointed to a highly competitive power of *H. pilosella* in its building and mature phases. Watt (1962) observed an apparent indifference of established *H. pilosella* to annual rainfall changes, and suggested that *H. pilosella* is a strong competitor for moisture. *H. pilosella* replaced *Festuca ovina*

L. particularly the weak, very young and very old plants (Watt, 1981). Watt also observed that the recovery of other species in formerly *H. pilosella* dominant grasslands appeared to be passive with *H. pilosella* degenerating first and the other species entering subsequently. In the early part of the 20th century, *H. pilosella* was mentioned as a minor weed of lower altitude British pastureland with suggestions that it displaced other vegetation (Long and Percival, 1910; Brenchley, 1920).

The "guerilla" or wandering strategy of stoloniferous species can leave them relatively more vulnerable to interspecific competition than species producing ramets with a tight phalanx strategy (Harper, 1978). The most successful control method for *H. pilosella* in New Zealand involves aiding interspecific competition from other species (Section I.3.4).

Another form of vegetative propagation is leaf axil buds close to the ground forming new rosettes. This has been reported in *H. floribundum* (Yeung and Peterson, 1971) and *H. pilosella* (personal observation), and can occur after seed set or senescence of the original rosettes leaves.

Hieracium spp. seed have a low percentage persistence in soil after one year and do not form a long term seedbank (Grime *et al.*, 1988; Panebianco and Willemsen, 1976). *H. pilosella* is poorly adapted to tall turf; Grime and Jeffrey (1965) showed that *H. pilosella* seedlings in the shade had a relatively low survival rate and produced negligible initial height growth. Grime *et al.* (1981) found that germination of *H. pilosella* seed was best in light though also occurred to a lesser extent in the dark (44% germination compared to 91%). The lesser germination in the dark indicates a poor adaptation to establishment in tall turf.

Seed germination of *H. pilosella* is often favoured by warm conditions (Grime *et al.*, 1981). Panebianco and Willemsen (1976) found that seed of *H. pratense* germinated best at relatively warm temperatures but that germination at lower temperatures (5 to 15°C) was enhanced by stratification. They interpreted this as an adaptation to habitats which are prone to severe winters. Germination would be low during cold conditions in

autumn when seedlings would have to overwinter, but after stratification from the winter, seed would germinate better in colder conditions of spring and able to become established before the stress of summer.

Stergios (1976) reported that achenes of *H. aurantiacum* were mostly deposited within one metre with achenes deposited near the source having a higher percentage viability and larger size than those deposited up to 3m from the source. Stergios (1976) found that cold treatment (stratification) increased germination of achenes collected towards the end of the growing season but not seed collected in mid-summer. This is likely to be a helpful adaptation for late produced achenes to overwinter dormant, and subsequently germinate in spring. Stergios (1976) pointed out that the achenes of *H. aurantiacum* were fulfilling the roles of saturating areas already invaded by the species, having some degree of dormancy to survive unfavourable conditions, and having an ability to locate suitable habitats by long-distance dispersal.

I.3.3.c Drought tolerance

Drought tolerance is likely to be a large component of the strategy of *H. pilosella* and is due in part to a prostrate rosette form which is subject to reduced air turbulence and high relative humidity (Grime and Jeffrey, 1965). Anderson (1927) described *H. pilosella* as having strong drought tolerance in British chalk grasslands despite having a shallow root system. Reader *et al.* (1993) demonstrated, however, that *H. pilosella* is capable of relatively deep rooting to 46 cm (mean depth) in drying soil during soil column experiments.

I.3.3.d Mineral uptake

H. pilosella has a strong capacity for mineral and other nutrient uptake which is likely to be a significant competitive advantage on soils of relatively low fertility. When comparing a range of plants with mycorrhizal infection, Grime *et al.* (1988) showed that transfer of C^{14} from *Festuca ovina* in microcosms was highest in *H. pilosella*, indicating a drain of resources from *F. ovina* to *H. pilosella*. The relatively high capacity for mineral uptake was further shown by *H. pilosella* being the only Otago

high country species found by Lee *et al.* (1992) to significantly accumulate arsenic. Lee *et al.* (loc. cit.) were investigating the potential of plants as indicators of gold bearing quartz veins. They found that *H. pilosella* accumulated up to 18 parts per million of arsenic, while tussock grasses contained under 2 ppm.

Mineral nutrient stress reduces plant growth directly and can lead to increased drought susceptibility of plants through reduced root penetration (Grime and Curtis, 1976).

I.3.3.e Herbivory

H. pilosella is not avoided by polyphagous invertebrate herbivores, in fact Grime *et al.* (1968) reported that palatability of *H. pilosella* was relatively high for the snail (*Cepaea nemoralis*). Snail herbivory is commonly observed on *H. pilosella* in Britain (personal observation). In a food aversion study with two polyphagous caterpillars (*Diacrisa virginica* and *Estigmene congrua*), Dethier (1980) found that *H. pilosella* was one of the most preferred of a range of species tested.

H. pilosella is also palatable to stock (Scott, 1984), but in open environments the prostrate stature of *H. pilosella* rosettes largely protects leaves and stolons from grazing damage. Grazing mammals often eat the flower stems and therefore affect the population dynamics of *H. pilosella* by preventing seed set and allowing the monocarpic rosettes to be perennial (Bishop and Davy, 1984).

I.3.3.f Allelopathy

Allelopathy occurs when a plant species releases growth inhibiting chemicals which affect neighbouring plants. There have been several reports on the possible allelopathic activity of subgenus *Pilosella* species. Widera (1978) proposed that allelopathy as a reason for the competitive ability of *H. pilosella* against *Festuca rubra* and other species in Poland, and Dawes and Maravolo (1973) saw allelopathy as a possible factor supporting the dominant role of *H. aurantiacum* in some USA bracken-grasslands.

There has however been no conclusive proof of the significance of an allelopathic effect of *Hieracium* species. Henn *et al.* (1988) found no significantly phytotoxic compounds in the soil under *H. pilosella* and, in experimental mixed cultures of *H. pilosella* and *Arrhenatherum elatius* (L.) J. & C. Presl., did not demonstrate any suppression of *A. elatius*. In New Zealand, studies have not demonstrated any importance of *H. pilosella* allelopathy in the field (Makepeace *et al.*, 1985; Orchard, 1994; Scott, 1975a). It may be possible that allelopathy has an effect at certain stages, for example a period of drought causing leaf senescence being followed by rain leaching the allelochemicals from leaves into the soil, but this effect is likely to be transitory (Scott, 1984). The levels of potential allelochemicals in *H. pilosella* have been shown to differ markedly by season (Duquénois *et al.*, 1956) and growing conditions (Adams, 1987).

I.3.4 CONTROL METHODS FOR HAWKWEEDS

Several control methods for *Hieracium* species in agricultural areas have been assessed by Scott (1990) including grazing, herbicide application, pasture improvement and biological control.

Grazing effectively reduces seed production but the habit of *H. pilosella* limits the intake of vegetative parts by sheep. Since the great majority of new rosettes of *H. pilosella* and *H. praealtum* come from stolons (Makepeace, 1985a), Scott (1990) suggested that *Hieracium* infested areas could be spelled during November and December to allow *H. pilosella* resources to go into seed production rather than vegetative propagation, thus reducing weed spread. *H. praealtum* has a more erect habit than *H. pilosella* and can be controlled to some extent by grazing (Scott, 1990).

Hieracium control with several herbicides was investigated by Makepeace (1985b) and Meeklah (1979). The general conclusion was that while acceptable levels of control were achieved with herbicides such as a mecoprop, MCPA and dicamba mixture, and 2,4,D ester, herbicide use over large areas of low productivity land was

of doubtful economical viability. A concern with the use of herbicide is that non-target dicotyledonous plants are also affected.

Fertiliser application and oversowing or overdrilling with pasture species has successfully controlled *H. pilosella* in some areas of the New Zealand high country. Scott *et al.* (1990) found that overdrilled legumes could suppress *H. pilosella* within two years after fertiliser addition on a moderately productive site. They found it took five to six years for suppression of *H. pilosella* to begin after similar overdrilling and fertiliser addition on a site of low productivity, showing that site factors influence the effectiveness of weed control through pasture improvement. *H. pilosella* may remain a component of the sward after fertiliser application and legume sowing (Scott and Covacevich, 1987) and so the treatment needs repeating after several years. Fertiliser applications were also found to reduce populations of *H. floribundum* in Canadian experiments (Reader, 1990). Reader (1990) interpreted the reduction in *H. floribundum* as resulting from increased competition from surrounding plant species because, when surrounding plants were removed, similar fertiliser treatments resulted in increased *H. floribundum* abundance.

The potential of non conventional pasture species such as tall oat grasses and sheep's burnet to grow in low fertility sites in the high country has been shown, but the ability to replace *H. pilosella* is uncertain (Scott, 1990).

I.3.5 NATIVE DISTRIBUTION OF *HIERACIUM*

Subgenus *Hieracium* species are native to the Northern Hemisphere; in Europe, northwestern Africa, northern and western Asia and North America, while the native range of subgenus *Pilosella* is confined to Eurasia and northwestern Africa (Sell, 1987).

H. pilosella is native to much of Europe except for Crete, Turkey, far northern areas of Scandinavia, and Iceland (Sell and West 1976; Bishop and Davy 1994). Other species of subgenus *Pilosella* are present in many of these regions barring the very far

north of Scandinavia. *Pilosella* species are also present in northwestern Africa. *H. caespitosum* is present in northern, central and eastern Europe. *H. praealtum* has naturalised in a wider range of European areas than *H. caespitosum*. The orange hawkweeds, *H. aurantiacum* and *H.* x *stoloniflorum*, have spread from mountainous areas in central and eastern Europe and both are now naturalised in most European countries, often as garden escapes.

I.3.6 CYTOLOGY OF EUROPEAN HIERACIA

Hieracium cytology has been intensively studied in Europe, where the majority of specimens were polyploid (Gadella, 1972, 1987; Skaliñska, 1967; Turesson, 1972; Turesson and Turesson, 1960). Often, variation was found in ploidy levels (number of sets of chromosomes per cell) within the one morphological species. For example, *H. pilosella* is represented in Europe by at least six ploidy levels from diploid (2X) through to heptaploid (7X). The ploidy level of *Hieracium* taxa is often an indicator of a breeding system, with odd ploidy levels commonly indicative of apomictic reproduction. Breeding systems can be either apomictic (seed forming from somatic division without meiosis and fertilisation), amphimictic (sexually reproducing) with self incompatibility, or apo-amphimictic (a combination of sexual and apomictic reproduction) (Gadella, 1987). Other aspects of *Hieracium* autecology, such as stolon length, can also be affected by ploidy levels (Gadella, 1987, 1991).

H. pilosella is mainly represented by the tetraploid cytotype in lowland Europe, and the pentaploid cytotype in northern Europe and montane areas. The British Isles and Scandinavia have a mixture of predominantly tetraploid and pentaploid cytotypes with pentaploids being generally more common in northern areas (Bishop and Davy, 1994; Gadella, 1992; Jalas and Pellinen, 1985; Turesson and Turesson, 1960). The hexaploid cytotype is found in lowland and montane areas but is not as common as the tetraploid and pentaploid cytotypes, while the heptaploid is even less common. Diploid and triploid cytotypes are scarce, but have been found in areas of the

European Alps (Gadella and Kliphuis, 1970; Delcourt, 1972) and in Breckland, southeast England (Misirdali in Bishop and Davy, 1994).

The Flora Europaea treatment of *Hieracium* by Sell and West (1976) lists recorded ploidy levels for European taxa. These records are presented in Table 1.3 with some additions.

H. pilosella was found to be represented by the pentaploid cytotype in New Zealand (Makepeace, 1981). Field collections of pentaploid *H. pilosella* in Europe are apomictic while the more common tetraploid race is generally sexually reproducing (Gadella, 1987; Turesson and Turesson, 1960). New Zealand pentaploid *H. pilosella* is also apomictic, though a small degree of sexual crossing cannot be ruled out (R. Bicknell pers. comm.). Pentaploid *H. pilosella* collections made in France were reported to have pollen with low percentage viability (Delcourt, 1972), but collections from Sweden were described as having good pollen (Turesson and Turesson, 1960). From pentaploid *H. pilosella*, Gadella (1987) reported some infertile pollen grains that did not appear to be full and others that were normal and demonstrated a good capacity to fertilise sexual tetraploid plants (Gadella, 1972;1987;1992). He also found that the pentaploid *H. pilosella* produced viable eu-diploid and eu-triploid pollen.

Gadella (1987) compared sexual tetraploid and apomictic pentaploid representatives in experimental garden conditions and found that in comparison with the tetraploid, the pentaploid produced longer and more numerous stolons with a greater ability to spread. He also reported that the pentaploid had relatively longer scapes and pentaploid rosettes produced a greater number of viable achenes per rosette. Previous work on Swedish *H. pilosella* (Turesson and Turesson, 1960) also found relatively longer scapes and stolons in the pentaploid when compared to the tetraploid. Gadella (1987) interpreted the differences as affording pentaploid *H. pilosella* a greater colonising ability. In steady wind conditions, increased height of scape gives a generally increased dispersal distance of an achene with a pappus (Sheldon and Burrows, 1973).

Table 1.3. Ploidy level records from European *Hieracium* taxa related to New Zealand naturalised Hieracia.
Data from Sell and West (1976) amended with additional counts (in brackets).

Species	Subspecies	Ploidy Levels
H. pilosella	combined	2X, (3X[b]), 4X, 5X, 6X, 7X, (9X[c]), (10X[c])
	micradenium[a] Naegeli & Peter	2X, 4X, 5X
	euronotum Naegeli & Peter	5X
	pilosella[a]	4X
	trichosoma[a] Peter	4X, 5X, 6X, 7X
	tricholepium Naegeli & Peter	4X
	melanops Peter	4X, 5X
	trichoscapum Naegeli & Peter	5X
	velutinum Fries	5X, 6X
H. praealtum	combined	4X, 5X
	praealtum[a]	4X, 5X
	anadenium (Naegeli & Peter) P.D. Sell	-
	bauhinii[a] (Besser) Petunnikov	5X
	thaumasium (Peter) P.D. Sell	-
H. caespitosum	combined	2X, 3X, 4X, 5X
	caespitosum[a]	5X
	colliniforme (Peter) P.D. Sell	-
	brevipilum (Naegeli & Peter) P.D. Sell	2X
H. aurantiacum	combined	2X, 3X, 4X, 5X, 6X, 7X, 8X
	aurantiacum	4X
	carpathicola Naegeli & Peter	-
H. x *stoloniflorum*		5X
H. argillaceum group		3X
H. lepidulum		no record
H. maculatum group[d]		no record
H. murorum group		3X, 4X
H. sabaudum L.		3X, 4X[e]

[a] Subspecies recorded in New Zealand (Garnock-Jones in Webb *et al.*, 1988).
[b] Data from Delcourt (1972) and H. Misirdali (in Bishop and Davy, 1994).
[c] Data from Gadella (1987). Plants with these ploidy levels were the result of glasshouse crosses, but the ploidy levels have not been recorded in the field.
[d] This group includes *H. pollichiae*.
[e] Referring to *H. sabaudum* L. (*sensu stricto*).

The pentaploid is the most common cytological race of *H. pilosella* in the northern reaches of Europe (Scotland and northern Scandinavia) and mountainous areas of the rest of Europe (Turesson and Turesson, 1960; Gadella, 1972), whereas the tetraploid is the most common in lowland central European areas (Gadella, 1972). The suitability of pentaploids to colder areas may be due to a higher nuclear DNA content. Bennett (1972) reported that all investigated plants with high nuclear DNA amounts had high minimum generation times of cell division. He noted that none of the high nuclear DNA plants were ephemeral. Plants with large genomes (e.g., high ploidy levels) may have a temporal separation of cell division (in suitably warm conditions) and cell enlargement which can occur in cold conditions not suitable for mitosis, leading to a general advantage over plants with smaller genomes (Grime and Mowforth, 1982). However, many authors have disputed the hypothesis of polyploids being physiologically favoured by extreme environments relative to lower ploidy levels of the same species (Pojar, 1973). An alternative explanation for the observed distribution of ploidy levels of some species would be that polyploids are comparatively favoured by sharp ecological gradients or frequent, drastic and irregularly occurring disturbances, presumably because of the greater potential genetic variability within a polyploid's nuclear material (Pojar, loc. cit.). In a review of electrophoretic studies, Soltis and Soltis (1993) presented strong evidence for genetic advantages in the potential success of autopolyploids in nature, relative to their diploid progenitors. These included enzyme multiplicity, increased heterozygosity and increased allelic diversity.

There have been few reports of aneuploidy (departures from strict multiples of the base number) in field collections of *Hieracium* taxa. *H. aurantiacum* was reported twice as 2n=30 in Eastern Europe (Zhukova, 1967; Pashuk, 1987). Ostenfield (1906) and Rosenberg (1908, 1917) worked with the same collection of *H. excellens* non J. Murr ex Zahn (subgenus *Pilosella*) that was reported by Rosenberg as 2n=42. The *H. excellens* collection was totally male sterile but could produce seed by apomictic reproduction and also sexually (as evidenced by hybrid progeny). Aneuploidy was reported in pollen of pentaploid *H. pilosella* by Gadella (1987) who found a

proportion of aneuploid plants among progeny of experimentally produced (by glasshouse crosses) sexual pentaploid mothers. Skaliñska (1971) discovered one weak aneuploid during a series of experimental crosses of *H. aurantiacum* cytotypes. A representative of *H. alpinum* group was recorded as 2n=26 by Sokolovskaja and Strelkova (1938). In Japan, a chromosome count of 2n=17 was reported for the subgenus *Hieracium* taxon *H. umbellatum* L. var. *japonicum* Hara. (Nisioka, 1958).

I.4 PATHOGENS OF *HIERACIUM*

A large number of pathogens are recorded on *Hieracium* in Europe and North America. This contrasts with a lack of pathogens on *Hieracium* taxa in New Zealand. Obligate fungal parasites of *Hieracium* in Europe are rust, powdery mildew, smut, and downy mildew. Details of these and facultative pathogens are given in Chapter VI. This section covers aspects of rust, which is presently the main candidate for fungal biological control of *Hieracium*.

I.4.1 RUST TAXONOMY

I.4.1.a General

The rusts are basidiomycete fungi from the order *Uredinales*. They have been strongly linked to their plant hosts throughout evolution because of their obligate parasitism. Rust taxonomy reflects this relationship; Savile (1970) emphasised the importance of host specialisation to be considered along with morphology, as a taxonomic criterion, with each rust taxon generally confined to a very limited host range. Rust taxa have been described as species on the basis of distinctive host range even without morphological variation. More recently though, it is less common for such taxa to be given species status, instead they are often assigned to subspecies, varieties or *formae speciales*. Even so, one of the main rust genera, *Puccinia* Pers., may contain 3000 to 4000 species (Hiratsuka and Sato, 1982).

For an understanding of rust taxonomy there needs to be an understanding of rust life cycles and morphology, as well as pathogenicity and resistance.

I.4.1.b Life cycle of rust

I.4.1.b(i) Stages of the life cycle

Rust fungi can produce up to five spore stages in succession. Species displaying all five stages are termed macrocyclic. In most macrocyclic fungi, urediniospores are the most common spore form during the summer period. The urediniospores are produced in sori termed uredinia and are asexual. Following germination and hyphal penetration of stomata of a suitable host, infection can produce a new uredinium. This stage serves as the main method with which most macrocyclic rust spread can progress. Often as winter time approaches, the uredinia of *Puccinia* spp. may begin to produce teliospores along with the urediniospores. When a sorus produces mostly teliospores it is termed a telium. Telia may also be produced in proximity to an old uredinium. Teliospores generally have a thicker wall.

The teliospore is often the form in which rust overwinters. In most species germination of a teliospore usually results in the production of a basidium bearing basidiospores. This involves karyogamy such that basidiospores are uninucleate.

Basidiospores may germinate in two fashions (Petersen, 1974), but most commonly the basidiospore produces a germ tube which infects host tissue. Infection leads to the production of haploid pycnia. Pycnia produce pycniospores and receptive hyphae of the same self-incompatible mating type. If a pycniospore contacts a receptive hypha of a pycnium of a compatible mating type, then fusion can take place and hyphae develop within host tissue to produce an aecium. Alternatively pycniospores can germinate on host tissue and produce hyphae (sometimes within host tissue) which may later fuse with compatible hyphae and give rise to aecia.

Standard aecia produce aeciospores bounded by a peridium. In *Puccinia* the aecia are often cupulate structures. Aeciospores germinate to produce germ tubes which penetrate host tissue through stomata, and subsequent infections give rise to uredinia.

Some rusts can overwinter in forms other than spores. In perennial or biennial plants, rust mycelium may survive in host tissue or, in mild conditions uredinia and telia may remain active. Hasan and Wapshere (1973) reported that *Puccinia chondrillina* apparently multiplied solely by the production of urediniospores in the Mediterranean region, even though teliospores were produced.

I.4.1.b(ii) Modifications of the life cycle

Rusts with a complete life cycle of all spore types are termed macrocyclic. If all five spore forms of a macrocyclic rust can be produced on a single host, the rust is termed autoecious. Macrocyclic rust species (for instance cereal stem rusts) that have alternate hosts with different forms of spores being produced on different hosts are termed heteroecious.

The potential of a heteroecious rust as a BCA may be limited by the need for an alternate host for part of the life cycle. Littlefield (1985) saw little prospect for the heteroecious rust, *Uromyces striatus* Schroeter as a BCA of leafy spurge in North America since the alternate host was *Medicago sativa* L., an important economic crop.

The majority of *Puccinia* species on Asteraceae are autoecious (Cunningham, 1931). There are no recorded heteroecious *Puccinia* spp. that produce the uredinial stage on Asteraceae, the only heteroecious rusts recorded on Asteraceae hosts being present in the aecidial stage.

Some rust species have spore and sori forms that do not adhere to the type forms described above. The terminology for some of these sori forms has been reviewed by Laundon (1973). The terminology for rust species exhibiting different life cycles and spore states was reviewed in Petersen (1974) from where Table 1.4 is taken.

Table 1.4 Terminology of rust types according to the presence and form of life cycle stages. (Taken from Petersen, 1974).

	Life Cycle Stages Present				
Rust Form	0	I	II	III	IV
Euform	±	+	+	+	+
Cataform	-	+	+	+	+
Brachyform	±	±[a]	+	+	+
Opsisform	±	+	-	+	+
Catopsisform	-	+	-	+	+
Microform	±	-	-	+	+
Hypoform	±	-	-	+	+
Leptoform	±	-	-	+[b]	+
Hemiform	-	-	+	+	+
Endoform	±	-	-	+[c]	+

The spore stages in the rust life cycle are given codes from 0 to IV. 0 represents pycniospores; I, aeciospores; II, urediniospores; III, teliospores; and IV, basidiospores.
'+' indicates the spore stage is present; '-', the stage is absent; and '±', the stage may be present.
[a] Aecia, if present, uredinoid, i.e. aecia similar in form to uredinia and aeciospores resembling urediniospores.
[b] Leptospores also produced.
[c] Teliospores are morphologically aeciospores.

I.4.2 *PUCCINIA HIERACII*

I.4.2.a Life Cycle of *Puccinia hieracii*

Puccinia hieracii, which infects *Hieracium* spp., is known to be macrocyclic and autoecious. *P. hieracii* has uredinoid aecia and therefore conforms to the brachyform description (see Table 1.4). Urediniospores are unicellular, echinulate and cinnamon brown. Teliospores are two celled, lightly verruculose, and darker brown than the urediniospores. Pycnia of *P. hieracii* on *Hieracium* are reported as yellowish clusters (Grove, 1913).

The timing of each of the spore stages for *P. hieracii* var. *piloselloidarum* on *H. pilosella* has been recorded by Wilson and Henderson (1966) in Britain. Pycnia and aecia were found to be present in May and uredinia and telia from June onwards. There are also some records of a type of teliospore, termed primary, within aecia of *P. hieracii* var. *hieracii* and var. *hypochoeridis* L. (Wilson and Henderson, 1966). Wilson and Henderson (1966) considered that the sometimes reported infrequency of

sexual stages of *P. hieracii* was likely due to overlooking the presence of aecia which resemble uredinia. The timing and occurrence of the rust stages are affected by local climate.

I.4.2.b Taxonomy of *Puccinia hieracii*

In Europe, *P. hieracii* consists of at least three distinguishable taxa. In some treatments the taxa have been described as species in their own right (Gäumann, 1959), or subspecies (Probst, 1909), or varieties (Wilson and Henderson, 1966).

The varieties are: *P. hieracii* var. *hieracii* (with a large host range including *Hieracium* subgenus *Hieracium*, other members of the Lactuceae, and *Centauria* L.); *P. hieracii* var. *piloselloidarum* (confined to *Hieracium* subgenus *Pilosella*); and *P. hieracii* var. *hypochoeridis* (Oud.) Jørstad (specific to *Hypochoeris* L. and *Crepis* L.). The urediniospores are morphologically distinguishable between varieties. Urediniospore germ pores are supraequatorial in var. *hieracii*, only slightly so in var. *piloselloidarum* and nearly or quite equatorial in var. *hypochoeridis* (Wilson and Henderson, 1966).

P. hieracii var. *hieracii* is present in New Zealand on the naturalised species *Cichorium intybus, Leontodon taraxacoides* (Villars) Mérat and *Taraxacum officinale* G. Weber (Pennycook, 1989), and is recorded on the indigenous *Microseris scapigera* (Sol. ex A. Cunn.) Sch. Bip. (McKenzie, 1981). There are no verified records of the rust variety on any of the adventive *Hieracium* subgenus *Hieracium* species. *P. hieracii* var. *hypochoeridis* is adventive to New Zealand on *Crepis vesicaria* L. and *Hypochoeris glabra* L. (Pennycook, 1989). *P. hieracii* varieties have been observed in the Mackenzie Country basin in the New Zealand high country on *Crepis* sp. and *Taraxacum officinale* (pers. obs.).

All three *P. hieracii* taxa are recorded in North America. Parmelee and Savile (1981) treated var. *hypochoeridis* on *Hypochoeris* L. as a species, *P. hypochaeridis* (*sic*) (= *P. hypochoeridis* Oud.). They treated *P. hieracii* var. *piloselloidarum* recorded on *H.*

pratense (=*H. caespitosum*), together with var. *hieracii* on a variety of hosts, as *Puccinia hieracii* with no varietal distinctions.

In experiments conducted on *P. hieracii* var. *piloselloidarum*, Probst (1909) described seven *formae speciales* based on their ability to infect a certain range of species and subspecies of *Hieracium*. The results of his attempts at cross infection are shown in Table 1.5. The original list of taxa that Probst experimented with has been interpreted in light of more recent *Hieracium* taxonomy. For instance *H. pratense* Tausch probably represents *H. caespitosum* Dumort. Most of the formae specialis that Probst described were only observed to infect their original host taxon. However, three of the formae specialis exhibited wider host ranges. Forma speciale *ziziani*, for instance, was able to infect *H. praealtum* and *H. caespitosum* as well as its original host, *H. zizianum*. Probst demonstrated differences between three rust isolates from *H. pilosella* such that only one of the isolates infected other species. He found that cross inoculations between two forms of *H. pilosella* ssp. *vulgare* were unsuccessful.

In the treatment of *Puccinia hieracii*, Grove (1913) described Probst's proposed *formae speciales* and races as biological races. Grove (p. 159) thought that Probst reduced "the question of such races to absurdity by 'proving' that one of them was restricted to a mere form of a variety of a subspecies" (*Hieracium pilosella*, subsp. *vulgare*, var. *genuinum*, forma *subpilosum*). Probst had only worked with a small subset of the variation present in subgenus *Pilosella* and in the species *H. pilosella*. Conclusions from the work of Probst are not of the complete host range of each of the races but rather that differences in host ranges apparently exist between the different rust races (or *formae speciales*). The cross infection experiments of Scott (pers. comm. 1995a), summarised in Table 1.7 (see Section I.4.7) demonstrate that isolates of *P. hieracii* var. *piloselloidarum* from several species could infect *H. pilosella*.

Table 1.5. The host range of *formae speciales* of *Puccinia hieracii* var. *piloselloidarum* described by Probst (1909).

HOST	*Formae speciales*[a]							
	A	B	C	D	Ea	Eb	F	G
H. aurantiacum L.		-	-	-	-		-	-
H. auricula L.	+	-	-	-	-	-		-
H. bauhinii Schultes	-	-	-	-		-	-	+
H. flagellare Willd.			-	-				
H. florentinum All.								
subsp. *alethes* N.P.		-	-	-	-		-	+
H. florentinum All.								
subsp. *obscurum* Rchb.		+						
H. hoppeanum Schultes			+		-			
H. peleterianum Mérat	+	-	-	+	-	-	-	-
H. pilosella L.								
subsp. *vulgare* a	-	-	-	-	+	-	-	-
b					-	+		-
c					-			
H. pilosella L.								
subsp. *velutinum* Heg. et Heer	-		-	-	-	-	+	-
H. pratense Tausch			-	-		-	-	+
H. rubrum Peter			-	-	-	+	-	-
H. sphaerocephalum Froel.				-	-	+	-	
H. stoloniferum W. et K.			-			-	-	
H. tardans Peter	-				-	+	-	-
H. zizianum Tausch		-			-			+

NOTE: a plus sign indicates positive infection, a minus sign denotes the lack of infection after an inoculation attempt.

[a] A: forma speciale *auriculae* Probst
B: f.sp. *florentini* Probst
C: f.sp. *hoppeani* Probst
D: f.sp. *peleteriani* Probst
Ea: f.sp. *pilosellae* Probst, race a
Eb: f.sp. *pilosellae* Probst, race b
F: f.sp. *velutini* Probst
G: f.sp. *ziziani* Probst

Many of the taxa names included in Probst's inoculation experiment have been replaced. Some of the name changes taken from Sell and West (1976) include
H. bauhini = *H. praealtum* Vill. subsp. *bauhinii* (Besser) Petunnikov
H. florentinum = *H. piloselloides* Vill. subsp. *piloselloides* P.D. Sell
H. pratense = *H. caespitosum* Dumort.
H. rubrum = *H.* x *rubrum* Peter (*H. aurantiacum* x *H. flagellare* Willd.)
H. stoloniferum may correspond to *H.* x *stoloniflorum* Waldst. and Kit.
H. tardans Peter was included in the *H. pilosella* complex by Sell and West, but retained its binomial.
H. zizianum = *H.* x *zizianum* Tausch (*H. cymosum* L. x *H. piloselloides* Vill.)

P. hieracii var. *hieracii* has an exceptionally wide host range relative to other *Puccinia* species on Lactuceae (31 species in eight genera in North America according to Parmelee and Savile, 1981). It is likely that within this variety there are many *formae speciales* with a narrower host range than the variety as a whole.

I.4.3 OTHER *PUCCINIA* SPECIES ON *HIERACIUM*

Three *Puccinia* species are known to be pathogenic on *Hieracium* species in North America (*P. hieracii, P. columbiense* Ell. & Ev., and *P. fraseri* Arth.) (Parmelee and Savile, 1981). A rust of the genus *Aecidium* is also recorded on *Hieracium* in North America (Cummins, 1978). *Puccinia hieracii* however is by far the most common on *Hieracium* and *P. hieracii* var. *piloselloidarum* is the only rust recorded on members of the subgenus *Pilosella.* None is recorded on New Zealand adventive *Hieracium.*

There are at least eighteen other species of *Puccinia* already present on Asteraceae hosts in New Zealand, infecting seventeen genera. Two of the species are present on introduced Lactuceae hosts, *P. crepidicola* H. & P. Sydow on *Crepis capillaris* (L.) Wallr. and *P. variabilis* Greville var. *lapsanae* (Fuckel) Cummins on *Lapsana communis* L.. Including those genera infected by *Puccinia hieracii*, there are altogether seven introduced Lactuceae genera recorded as hosts of *Puccinia* rust in this country.

Considering how common *Hieracium* is, and that the other most common weed genera of the Lactuceae host their associated rust pathogens, it is curious that rusts attacking *Hieracium* are not present. The rusts recorded on *Taraxacum* and *Crepis* in New Zealand are apparently only recent introductions (E. McKenzie pers. comm.).

I.4.4 RUST UREDINIOSPORES

Most work on *Puccinia hieracii* pathogenicity has involved uredinia and urediniospores (Probst, 1909, Scott, pers. comm. 1995a; and the current work). This section outlines some of the knowledge of the physiology and action of *Puccinia* urediniospores and the subsequent infection process.

I.4.4.a Infection process

There are very few reports of *Puccinia* penetrating tissue directly. As a rule, stomatal openings are necessary for penetration of the host. Urediniospores germinate to produce a germ tube which forms an appresorium above a stoma. Hyphal growth from the appresorium then penetrates the host tissue through the stoma and then follows a period of intercellular mycelial growth. To obtain resources from the host plant, the rust forms haustorial mother cells, which produce haustoria penetrating the host cell wall, leaving the host cell membrane intact.

Rust hyphae form compacted areas with a series of hyphal tips in a palisade layer. Urediniospores are produced from the hyphal tips. In *Puccinia* this occurs just beneath the host epidermis. The pressure of urediniospore production and hyphal growth cause the host surface to rupture and the sori are exposed and continue to produce spores. Once dispersed, the urediniospores can give rise to further uredinia.

I.4.4.b Dispersal of urediniospores

Wind dispersal is an important mechanism for the transfer of rust urediniospores (Manners, 1981). Rain splash may also be effective in dispersal of urediniospores. This has for instance been demonstrated for *P. striiformis* Westendorp (Rapilly *et al.*, 1970). The existence and extent of effective invertebrate dispersal is uncertain. Circumstantial evidence of a weevil aiding dispersal of *Puccinia punctiformis* (rust of *Cirsium arvense*) was presented by Peschken and Beecher (1973). They found that the level of rust infections were higher where the insect was present.

I.4.4.c Factors affecting germination and infection

Some species of *Puccinia* produce highly variable qualities (in terms of ability to infect) of urediniospores depending on the conditions in which they have been produced, for example *P. striiformis* (Manners, 1981). Some of the factors that affect germination and subsequent infection are detailed below. Lucas and Knights (1987)

considered that external biotic factors did not appear to greatly affect urediniospore germination and that the physical environment was more significant.

I.4.4.c(i) Age of spores

The age of urediniospores can affect the percentage germination and the ability to cause infection. In a matter of months or less, urediniospores may be too old for optimum germination and infectivity. The longevity of urediniospores of crown rust was found to be dependant on the environmental factors of temperature and humidity (Rosen and Weetman, 1940). Urediniospores may also be too young; Eversmeyer and Kramer (1989) working with urediniospores of *P. recondita* Roberge ex Desmazieres found increased germination percentages when harvested by vacuum (selecting "mature spores") than when collected directly, implying that a proportion of the spores present in the sori were immature.

I.4.4.c(ii) Temperature

The optimum temperature for urediniospore germination may vary according to species, variety, *formae speciales* and even physiological race, but generally the optimum is between 10°C and 25°C (Manners, 1981). At temperatures too low or too high, germination percentage can be lowered and germ tube extension reduced and more coiled rather than straight (e.g., Kramer and Eversmeyer, 1992). Park (1990) found that the percentage of wheat seedlings infected 15 days after inoculation with *P. striiformis* f.sp. *tritici* decreased with higher incubation temperatures (0% infection at 21.4°C). The optimum temperature for infection by *P. striiformis* f.sp. *tritici* was 15.4°C.

The latent period (period between inoculation and eruption of sori) of rusts can also be affected by temperature. The latent period of *Puccinia arachidis* Speg. for production of uredinia on groundnut (*Arachis hypogaea* L.) was found to range from 12 to 49 days, largely dependent on temperature (Wadia and Butler, 1994); low temperatures caused the longest latent periods and there was a small increase in latent period with higher than optimum temperatures. The latent period of *P. chondrillina* was found to vary from six days at 30°C to 43 days at 5 °C (Hasan and Jenkins, 1972).

The temperature during urediniospore production can markedly affect the temperature requirements for germination (Park, 1990). Straib (1940) found that urediniospores of *P. striiformis* produced at 20-25 °C had a maximum temperature of germination 2-3 °C higher than that of spores produced at 8-12 °C.

I.4.4.c(iii) Moisture

Sharp *et al.* (1958) showed that *P. graminis* Persoon urediniospores required liquid water for germination; Manners (1981) considers that this is probably true of other rust urediniospores. The length of "dew period" (a time of high moisture availability suitable for spore germination and germ tube extension) necessary for successful infection varies between rust taxa.

I.4.4.c(iv) Light

Some degree of photodormancy has often been noted in rust urediniospores (Lucas and Knights, 1987). In such cases, light delays germination rather than imposes an indefinite dormancy and Lucas and Knights interpreted the effect as probably helping to synchronise urediniospore germination with night and hence lower danger of desiccation. The germination, germ tube growth and appresorial formation of *Puccinia coronata* Corda var. *avenae* and *P. graminis* f.sp. *avenae* urediniospores have been shown to be optimal in darkness (Kochman and Brown, 1976). Burrage (1970) considered that the effects of light on *P. graminis* urediniospore germination were usually less than the effects of temperature.

The effect of light in triggering stomatal opening may have some effect on rust infection. Burrage (1970) reported that *P. graminis* urediniospores were able to infect during incubation in darkness but that the number of pustules formed increased with longer hours of light per day of incubation.

I.4.4.c(v) Chemicals

The presence of sugars, yeast extract and certain mineral salts have been shown to stimulate germination and germ tube growth of urediniospores *in vitro* (Straib, 1940).

The work of Scott (pers. comm. 1995a) indicated a depressive effect of sugars on *in vitro* germination of *P. hieracii* var. *piloselloidarum*.

I.4.4.c(vi) Self inhibitors and self stimulants

An optimum density of urediniospores exists for *P. graminis* and other rusts (Manners, 1981). Van Sumere *et al.* (1957) proposed that with increasing density of urediniospores, concentrations of stimulants favour germination up to the optimum density after which increasing concentrations of inhibitors reduce germination. Some inhibitory and stimulatory chemicals of urediniospores have been identified for several rust fungi (Allen, 1976; Manners, 1981).

I.4.4.c(vii) Microorganisms

Contamination with bacteria and fungal spores can affect the results of physiological experiments with urediniospores (Staples and Wynn, 1965). Manners (1981) reviewed the effects of various fungal and bacterial species on rust urediniospores, and he presented several examples of antagonism of spore germination and rust infection. Some bacteria have shown potential as BCAs of rust, for example *Bacillus cereus* to control *Puccinia allii* Rudoolphi on leek (*Allium porrum* L.) (Doherty and Preece, 1978).

I.4.4.c(viii) Host condition - spore production

Staples and Wynn (1965) state that the growth conditions of host plants could have an effect on the quality of urediniospores, potentially affecting experimental results. Gopalan (1980) reported that urediniospores of *P. striiformis* produced on senescent leaves gave inferior germination percentages compared to those produced on healthy leaves. Considering these effects of host condition, it seems possible that the genotype of the original host has an effect on the urediniospores produced by a rust.

I.4.4.c(ix) Host condition - initial infection

The symptoms of infection on an inoculated host are dependent on the presence of susceptibility or resistance to the rust pathogen. This can be genetically controlled as discussed in Section I.4.5 below, and physiological condition of the plant may also

have an effect. Mortensen (1985a) reported that *P. jaceae* Otth., a rust pathogen of *Centaurea* spp. L., could infect safflower (*Carthamus tinctorius* L.) but only in the seedling stage. The cotyledon leaves were fully susceptible and the first true leaves displayed hypersensitivity but all other leaves were not infected by rust.

Trapping efficiency of host tissue can affect rust infection, for example Rapilly and Fourcault (1976) found that *P. striiformis* urediniospores were deposited less on hairy leaves. Russell (1975) reported that more spores were deposited on one wheat cultivar than two other cultivars, under similar conditions; the difference was attributed to the leaves of the former cultivar being more vertical.

Plant disease is a result of the interaction between a susceptible host plant, a pathogenic organism and environmental factors. It is possible for environmental factors to affect the susceptibility of a host. A link between high levels of available soil nitrogen and increased susceptibility of plants to rust infection has often been noted (Yarwood, 1976). West (1995) reported that rust (*Puccinia lagenophorae* Cooke) infection did not become established on phosphorus-limited *Senecio vulgaris* L. plants.

Yarwood (1976) discussed predisposition of hosts to either susceptibility or resistance (i.e. induced susceptibility or resistance) to a pathogen. In some instances, environmental factors may cause a lesser degree of susceptibility, for example Sharp (1965) found that wheat grown at 24°C before *Puccinia* inoculation was more or less susceptible (differing between cultivars) than wheat grown at 15°C. Daly *et al.* (1970) demonstrated that wheat with a dominant Sr6 allele for resistance to wheat stem rust was resistant at 20°C and susceptible at 26°C. Higher light intensity may increase, decrease or not change infectivity, depending on the species of rust and host (Kochman and Brown, 1975).

I.4.5 COMPATIBILITY OF PLANT AND PATHOGEN

Heath (1981) reviewed the resistance reactions of plants to rust fungi, and described six main defence mechanisms (Table 1.6) as evidenced by cytological investigations. She pointed to the distinction between the resistance reactions of "host" plants, which was generally reaction number 6, and the reaction of "non-host" plants, generally one or more of reactions 1 to 5. She also suggested that the host range of a pathogen may be determined more by the ability of the rust to overcome the pre-haustorial (reactions 1 to 5) defences of a plant than by the compatibility of the fungus and plant cells in a metabolic relationship. Heath (1981) noted that reports of inhibition of germination (reaction 1) were rare.

Wynn (1976) observed bean rust, *Uromyces phaseoli* var. *typica* (Pers.)Wint. on replicas and actual leaves of graminaceous plants. He found that less than 5% of germinated urediniospores formed appresoria in comparison to at least 92% on both beans (*Phaseolus vulgaris* L. 'Pinto') and replicas, indicating at least a partial reaction number 2.

Table 1.6. Plant defence reactions to rust fungi.

Reaction Number[a]	Description of Defence Reaction
1	- inhibition of germination
2	- topographical differences to host tissue such that germ tubes fail to find stomata
3	- physical or chemical barriers to tissue penetration
4	- inhibition of infection structure growth (inherent, or induced by rust)
5	- inhibition of haustorium function (degradation of haustorial mother cell walls, or deposition on plant walls of silicon-rich material)
6	- reduction of growth after formation of the first haustorium (e.g., incompatibility of the metabolic relationship between plant and rust, poor haustorial function, necrosis of plant cells, or phytoalexin release by the plant)

[a] Described by Heath (1981).

Phytoalexins have often been implicated in resistance reactions, but Darvill and Albersheim (1984) pointed out that while phytoalexins are important for resistance, they are but one of a number of disease resistance mechanisms in plants.

I.4.6 RUST EPIDEMIOLOGY

Scott (pers. comm. 1995a) described *P. hieracii* var. *piloselloidarum* on *H. pilosella* as an endemic pathogen (in the sense of Van der Plank, 1975). An endemic pathogen is characterised by a relatively stable population size compared to an epidemic pathogen that exhibits dramatic increases in population. *P. hieracii* var. *piloselloidarum* is widespread throughout the native range of *H. pilosella* but severe rust infestations were not commonly observed during the field studies of Scott (pers. comm. 1995a). Endemic pathogens can exert an influence on host vigour, but over a relatively long time rather than a short term severe effect of an epidemic pathogen. Endemic rusts may in certain years, given favourable conditions, severely damage susceptible hosts for a part of the season (Segal *et al.*, 1980).

I.4.7 EFFECT OF RUST ON HOST PLANTS

Pathogens may have a significant effect on populations of a plant species without being conspicuous or appearing to cause damage (Augspurger, 1989). The effect of pathogens can be direct and can exacerbate other stresses.

Direct effects of rust disease on plant growth can be caused by the sink of plant metabolites and the reduction in photosynthetic area. Observations on the translocation of radioactively labelled carbon (Livne and Daly, 1966) indicated that rust infected leaves of bean plants accumulated considerable proportions of the carbon, to the probable detriment of other leaves, including young emerging leaves. Ahmad *et al.* (1982) reported that phosphorus concentration in uninfected leaves was reduced on rusted barley. On healthy plants, growing zones may receive translocated nutrients from old senescing leaves, but Finney (1979) thought that powdery mildew

on leaves of barley might restrict that important process. Rust infections on senescing leaves may have a similar effect on redistribution of nutrients.

Infection with a plant pathogen may result in increased effect of abiotic and biotic stress on host plants (Ayres, 1984). Ayres made the point that abiotic stress often affects the whole plant and thus may reduce the ability of a plant to produce compensatory growth in response to pathogens. Rust disease can predispose a host plant to environmental and other stresses, either through reduced vigour or because of sori rupturing of the leaf cuticle and epidermis. Water deficits can occur in plants during dry periods and also in winter periods when soil water is frozen (Ayres and Paul, 1986). Rupturing of leaf tissue epidermis and cuticle by rust sori reduces leaf resistance to water loss (Owera *et al.*, 1981). Host mortality and damage from winter conditions can be exacerbated by rust infection due to susceptibility to injury earlier in the winter and reduced tolerance of winter stresses (Paul and Ayres, 1986).

Rupturing of the leaf epidermis by sori can also allow infection by other fungi and bacteria, in some cases causing severe reductions in host vigour and even host death (Hallett *et al.*, 1990). An integration of classical and inundative biological control may be possible. Hallett *et al.* (1990) reported inoculation with conidia of *Botrytis cinerea* (suspension of 1.24×10^6), killed all treated *Senecio vulgaris* plants previously infected with rust (*Puccinia lagenophorae* Cooke). *B. cinerea* entered rust sori and infected plant tissue, which often led to *Botrytis* growth into stem bases.

The water balance of a rust infected plant may be negatively affected by reduced root growth and ability to take up water as well as ruptured leaf tissue (Ayres and Paul, 1986). Tissera and Ayres (1986) found that rust (*Uromyces viciae-fabae* (Persoon) Schroeter) infection restricted the growth of *Vicia faba* L. roots down unwatered soil columns. Supported by experimental work with *Puccinia lagenophorae* on *Senecio vulgaris*, Paul and Ayres (1987) saw water stress as playing an important role in the impact of rust on mixed populations of plants in the field, with rust expected to have greatest effect on plant growth when water is limiting. C. Berryman and J.F. Farrar are reported in Farrar and Lewis (1987) as showing lowered water potential in brown

rust-infected barley leaves as leaf water content and status decline. Particularly severe effects of rust on vegetative growth under conditions of drought have been shown in wheat (Van der Wal *et al.*, 1975) and sunflower (Siddiqui, 1980).

The effect of the rust *Puccinia chondrillina* and competition from sub-clover (*Trifolium subterraneum* L.) on the growth of *C. juncea* was greater than either the effect of the plant or of the rust (Groves and Williams, 1975).

Foliar pathogens have been shown to cause premature senescence of leaves, e.g., powdery mildew of barley (Finney, 1979) and many rust species (e.g., Parker *et al.*, 1994; McKenzie and Hudson, 1976).

There have been several reports of the effect of pathogens on plants stressed by overwintering, for example root rot pathogens including *Bipolaris sorokiniana* (Saccardo) Shoemaker have been shown to reduce winter survival of wheat and barley (Frank, 1985). Frank's experiments were conducted in Pennsylvania where stand reductions attributed to *B. sorokiniana* could be 11 to 29% in wheat and 27 to 62% in barley. Paul and Ayres (1991) found that *Puccinia lagenophorae* reduced the resistance of *Senecio vulgaris* tissue to freezing and inhibited the tolerance of freezing damage.

There is much circumstantial evidence that plant pathogens play a significant role in plant community structure (Chilvers and Brittain, 1972; Burdon and Jarosz, 1988). Dinoor and Eshed (1984) suggest that in natural habitats, a state of dynamic equilibrium may exist between host populations and low levels (sometimes barely discernible) of pathogen. Gates *et al.* (1986) state that a host specific pathogen attack could affect the competitive relationship of susceptible plants with resistant ones. This has been supported by observations of, and experiments with, the effect of plant pathogens including rust, on intra- and interspecific competition (Burdon, 1980; Burdon and Chilvers, 1977; Burdon *et al.*, 1984).

I.5 PREVIOUS WORK ON FUNGAL BIOLOGICAL CONTROL OF *HIERACIUM*

I.5.1.a Introduction

Preliminary research on fungal biological control of *Hieracium* was conducted by Scott (pers. comm. 1995a). Scott experimented with rust and powdery mildew (*Erysiphe cichoracearum* DC.) of *Hieracium* spp., collecting over 70 rust isolates (*Puccinia hieracii* var. *piloselloidarum*) from throughout Britain and Scandinavia and testing them on New Zealand *Hieracium* plants and detached leaves. He investigated the pathogenicity and host range of the pathogens. The following sections summarise the findings of Scott (pers. comm. 1995a).

I.5.1.b Host Range of Rust

Scott showed that the range of host species infected by the rust included *H. pilosella*, *H. praealtum* and *H.* x *stoloniflorum*. *H. caespitosum* was never infected with the rust. Preliminary host specificity tests showed no sign of infection on non-target species. Table 1.7 summarises the results of Scott's (pers. comm. 1995a) attempts at cross infection between *Hieracium* hosts.

Table 1.7. Cross infection of *Puccinia hieracii* var. *piloselloidarum* on species of subgenus *Pilosella* (values = times infective_times tested).

	Inoculated Host			
Source of Rust	*H. pilosella*	*H. praealtum*	*H.* x *stoloniflorum*	*H. caespitosum*
H. pilosella	120_480	12_224	12_178	0_105
H. praealtum	2_20	1_6	0_5	not tested
H. flagellare	3_14	0_6	0_1	0_4

I.5.1.c Pathogenicity of Rust

The rust was observed on field populations of *H. pilosella* in Britain, Sweden and Norway. Levels of rust infection on *H. pilosella* were reported to be generally low. It was also postulated that the rust had its highest incidence in the spring and autumn and results of experimental infections with the rust in the summer were poorer than in

spring. There were just two reported incidences, both in Scandinavia, of rust infections affecting all leaves and stolons, evidently deforming the rosettes. When the two sites were observed in mid summer in a subsequent year, the infections were not severe. In all other observations the rust was perceived as occurring at low levels. This was in terms of the number of populations affected (5-10% of those investigated), the percentage of rosettes infected in a patch where the rust was present (about 1 to 2%) and the perceived severity on infected rosettes. The plants most infected appeared to be ones stressed by other factors, particularly waterlogging, competition and disturbance.

Many of the glasshouse infectivity experiments were carried out on detached leaves kept on water agar (1%) with 40ppm benzimidazole. The selected leaves were first or second fully expanded leaves. Detached leaves could last for longer than four weeks in these conditions in petri dishes. Inoculations with the rust fungi only resulted in percentage infections that varied over time. Percentage infection of leaves in late spring (April-May) was 15-(34)-43% [minimum-(mean)-maximum], in early summer 5-(31)-77% and in late summer just 0-(17)-38%. These differences were reported to be unrelated to glasshouse temperatures. In early autumn (September), infection of leaves and plants in the glasshouse was poor, as was sporulation on infected plants.

On average, only 17% of plants inoculated became infected. Generally infection was limited to just a few leaves on each plant successfully infected. Usually an infection from one spore resulted in a single sorus. However there were some cases when composite sori formed, apparently through limited intercellular spread of mycelium.

The reported latent period for the rust was generally between 2-3 weeks. There was no evidence of longer latent periods which might have indicated a dormant or quiescent stage.

A small series of experiments was conducted in the glasshouse to elucidate the direct effect of growth on New Zealand *H. pilosella*. Eight weeks after inoculation the rust gave an apparent growth rate reduction of c. 2.7% per log(sori+1). An investigation

of the effect on New Zealand *H.* x *stoloniflorum* yielded an estimated growth rate reduction of 7.2% per log(sori+1).

I.5.1.d Urediniospore Experiments

Scott (pers. comm. 1995a) carried out a series of studies on environmental effects on urediniospore germination and rust infection. The spores were hydrophobic and when in large water droplets on a host leaf, gravitated toward the lower section and the midrib. Spore germination was tested in petri dishes with spores being deposited more or less evenly with a cotton bud onto moist filter paper placed on water agar. Under some conditions, spore germination resulted in germ tubes greater than 5-10 spore diameters (ca.100-200μm) within a day. Even under suitable conditions spore germination was generally in the 30-60% range. At different times spores of the same isolate had differing percentages of germination under similar conditions.

Spore germination was reduced by an increase in moisture tension. The optimum temperature for spore germination, in terms of the proportion of spores germinating, was estimated to be in the 14° to 18°C range. The presence of low aeration water (cooled, boiled, distilled water) increased levels of urediniospore germination. An apparent gaseous interaction from host leaves affected spore germination in petri dishes; the effect was sometimes promotory and sometimes inhibitory. The main gas was presumed to be ethylene. Carbon dioxide replacement of atmosphere in petri dishes severely depressed germination while nitrogen gas had a variable effect.

Germination following two hours immersion in water of various temperatures showed that exposure to high temperatures resulted in reduced a reduced level of spore germination. Brief exposure to low temperature as well as brief freezing was found to promote germination.

Spores stored in liquid nitrogen without the use of cryogen retained infectivity. Spores stored in the fridge or freezer for up to two months also resulted in successful infections.

I.5.1.e Powdery Mildew

Powdery mildew attack occurred in the glasshouse and caused severe infections on New Zealand *H. pilosella*. In limited host specificity tests the powdery mildew was found to infect a New Zealand endemic Lactuceae plant, *Kirkianella novae-zelandeae* (Hook. f.) Allan.

I.5.1.f Implications

The implications from this exploratory work of Scott (pers. comm. 1995a) was that the following aspects needed further investigation in exploring the potential of fungal biological control of *Hieracium*.

I.5.1.f(i) Rust

Johnston (1990) discussed the potential for fungal biological control of *Hieracium*, as part of a review of the scope for fungal biological control of important New Zealand weeds. This discussion on *Hieracium* was based on access to unpublished studies of Scott (pers. comm. 1995a). Johnston suggested that further work on the rust pathogen would require investigations of the suitable conditions for disease development and a search for more damaging strains of the rust.

Further research was also desirable on rust infectivity on a larger range of genotypes of *H. pilosella*, the biology of the rust, and the factors affecting rust presence and severity in the field.

I.5.1.f(ii) Powdery mildew

Further work should involve host specificity testing on the powdery mildew isolates collected.

I.6 STUDY OUTLINE

I.6.1 OBJECTIVES

The aim of the thesis was to investigate the potential for fungal biological control of *Hieracium* with particular emphasis on rust. A primary objective was to collect and screen a large number of rust isolates from a wide geographical range of sites.

The practical aims involved the screening of rust isolates to identify those most pathogenic on New Zealand *H. pilosella* and to prepare for the introduction of *Hieracium* rust to New Zealand. Investigations into rust biology and host specificity were therefore of great importance.

I.6.2 OUTLINE OF THESIS

Previous work on *Hieracium* biological control (Section I.5) included research on a rust pathogen, *Puccinia hieracii* var. *piloselloidarum*, and a powdery mildew, *Erysiphe cichoracearum*. This thesis concentrates on the *Puccinia* rust. Powdery mildew and other pathogens were also observed in the field and in glasshouse conditions and some observations on these are included.

The assessment for the potential of fungal biological control was focussed primarily on *H. pilosella*, which is recognised as the most significant *Hieracium* weed in New Zealand. Information on the field occurrence of *Hieracium* pathogens was gathered over a wide geographical range in Europe. The pathogen biology was studied in the field, glasshouse and laboratory, and factors affecting pathogenicity were investigated. Host taxonomic work and general study of the biology of the host weeds was undertaken. The relationship between variants present in New Zealand *Hieracium* and their susceptibility to rust isolates was studied.

The collection and propagation of host and rust collections are recorded in Chapter II. Chapter III deals with observations and experiments with the rust pathogen that were carried out in the glasshouse and laboratory. Field studies of rust were also conducted and are reported in Chapter IV. Chapter V covers the screening of over 1500 rust

isolates on New Zealand *H. pilosella*. The observations of other pathogens of *Hieracium* are summarised in Chapter VI. Research on genetic variation in New Zealand *Hieracium* taxa included the taxonomic methods of chromosome counting and isozyme electrophoresis (see Chapter VII). The general discussion and conclusion in Chapter VIII includes an assessment of the likely effect of fungal biological control and the implications of this research for the implementation of biological control.

Preliminary work was based at Canterbury University, in the Plant and Microbial Sciences Department and, for the isozyme electrophoresis pilot study, in the School of Forestry. Most experimental work was conducted at the Royal Botanic Garden, Edinburgh, U.K, with an additional five month period based at the Commonwealth, Scientific and Industrial Research Organisation (CSIRO) Unit of Biological Control, Montpellier, France.

Chapter II: HOST AND RUST COLLECTIONS

II.1 FIELD COLLECTION OF RUST

Collections were made in several regions of Europe. Most collections were made during cycle tours around the various regions in 1993 and consequently most isolates were collected from roadside areas. The abundance of rust infections on *H. pilosella* at each site visited was recorded, based on a visual estimate of the percentage of plants infected at the site (Table 2.1). Independent plants were defined as rosettes of *H. pilosella* that had a root system. Each of 1482 rust infected sites were sampled for rust isolate collections. In some cases more than one isolate was taken from a site, either because of morphological differences between infected plants of *H. pilosella* patches within the site, or for investigating within site variation. In total, 1434 of these rust isolates were subsequently tested for pathogenicity on New Zealand plants or detached leaves.

Infection level was assessed visually at each site in terms of percent patches with rust sori and percent rosettes within patches bearing sori. No assessment was made at 26 British sites from which isolates had been collected by colleagues. Rust infection levels were generally greater in the spring with a secondary peak of infection in the autumn. In the Jura area, perhaps due to high atmospheric moisture levels and rainfall, there was still a high percentage of sites, and rosettes within sites, with rust infection present in summer. In most other areas it appeared that there was a marked reduction in visible rust infection levels during the summer. This would have been a reason for the apparent low levels of rust infection seen in regions which were surveyed in mid-summer.

Table 2.1. Levels of rust infections observed during field collection of European isolates.

REGION	NO. OF SITES OBSERVED	NO. OF SITES WITH RUST	% SITES WITH RUST	MEAN % PLANTS INFECTED[a]	MAX % PLANTS AFFECTED IN A PATCH	MONTH OF OBSERVATIONS
Ireland	217	181	83	28	100	Mar.,Apr.
Britain	5306	1073	20	25	100	Feb.,Mar.,Apr., June to Nov.
Sweden	c. 1000[b]	12	c.1.2	3	60	May
N. Germany[c]	652[b]	4	0.6	12	50	May
S. Germany	164[b]	9	5	12	25	late May
Swiss (Jura)	188[b]	134	71	46	100	late May,early June
French Alps	824[b]	54	7	14	80	early June
Italian Alps	53[b]	12	23	5	15	early June
Belgium	35	0	0	-	0	early June
Denmark	41[b]	3	7	4	5	May

[a] Visual estimate. Mean CV (standard deviation / mean) for the mean percentage of plants infected was 1.4.

[b] Most rust isolates failed to survive on their host plants during postage (see Section II.2.2), a proportion of isolates were also collected as urediniospores on cotton buds and survived transport.

[c] Northern Germany and the north coast of the Netherlands also.

The Pyrenees region in the vicinity of Lourdes and surrounding mountains was surveyed in late May (early summer) 1992. Climatic conditions were hot and dry and the approximately 200 sites of *H. pilosella* observed were free of visible rust infection. On return to the area in October (autumn) 1993, observations were made at two of the largest *H. pilosella* populations previously surveyed. Rust infected plants were present in all patches of both populations, 40-60% of rosettes had uredinia and the occasional rosette had severe rust infections. Six further isolates of rust were collected from other parts of the mid to east Pyrenees in October, 1993, from a total of ca. 40 sites investigated.

II.2 RUST COLLECTION

II.2.1 METHODS OF COLLECTION

Rust isolates were collected by transplanting infected plants from the British Isles and throughout northern and central Europe to Edinburgh glasshouses. If plants were collected in the field during a tour outside of Britain they were sent by postage to the quarantine house of the Royal Botanic Garden, Edinburgh, where they were inspected and potted up.

Survival of plants and rust isolates was highly successful if the post took less than ten days. The postage time from most continental European countries was slow, with some consignments taking over three weeks to arrive in Edinburgh. In most cases the rust isolates on Continental hosts did not survive this period due to leaf senescence or heavy secondary infection (facultative pathogens infecting leaf tissue through the rust sori) on most rust sori. Plants with the heaviest rust infections were the least likely to survive mailing because of severe secondary pathogen invasion.

After notification from Edinburgh of the poor condition of the first consignment of European plants, some isolates from continental Europe were also collected by wiping infected leaves in the field with a slightly moist cotton bud, to collect urediniospores. The cotton bud was allowed to dry and a collection of cotton buds, kept separate by paper and card, was posted to Edinburgh.

II.2.2 RUST ISOLATES COLLECTED FROM THROUGHOUT EUROPE

Isolates detailed in Tables 2.2-2.4 are listed in decreasing order of infectivity during subsequent screening procedures on New Zealand *H. pilosella*. The top five ranked isolates, for infectivity on New Zealand *H. pilosella*, were all collected from Ireland (Table 2.2). The list of British isolates Table 2.3 is in order of infectivity in a limited screening procedure (Section V.3.4). Two isolates from France and one from Sweden (Table 2.4) demonstrated the most infectivity on New Zealand *H. pilosella* of all the continental European collections. All the rust isolates detailed below were collected from *H. pilosella* hosts. Isolates of *Puccinia hieracii* var. *piloselloidarum* were also collected from *H.* x *stoloniflorum* (1 isolate), *H. praealtum* (6) and *H. flagellarum* (7). Isolates of *P. hieracii* var. *hieracii* were collected from *H. murorum* agg. (13 isolates), *H. sabaudum* agg. (3), and *H. sylvaticum* agg. (1). Some miscellaneous rust isolates used in the present study but not necessarily highly ranking are given in Table 2.5.

Table 2.2. Most infective rust isolates from Ireland.

Isolate Code	Grid Co-ordinate	Origin
ÉIRE14	52°16'N 7°06'W	SW of Slieveroe, County Kilkenny
ÉIRE32	51°52'N 8°28'W	S of Cork, County Cork
ÉIRE29	51°47'N 8°29'W	Riverstick, County Cork
ÉIRE45	51°58'N 9°36'W	Upper Lake, County Kerry
ÉIRE66	51°58'N 9°36'W	Mitchelstown, County Cork
ÉIRE4	52°65'N 6°31'W	Upper Lake, County Kerry

Table 2.3. Most infective rust isolates from Britian, initial screening (Section V.3.4)

Isolate Code	Grid Co-ordinate (British OS Maps)[a]	Origin
BRIT12	NT 2772	Innocent Railwayline (I) , Scotland
BRIT21	NH 8425	Slochd I, Scotland
BRIT330	NT 1350	SW of West Linton, Scotland
BRIT627	NY 3488	NW of Langholm, Scotland
BRIT341	NS 9440	SE of Larnack, Scotland
BRIT1120	NZ 0697	NW of Forestburn Gate, Scotland
BRIT429	NS 9427	Roberton, Scotland
BRIT170	NN 8200	NE of Stirling, Scotland
BRIT278	NI 9620	W of Cullachie, Scotland
BRIT397	NX 3968	Challoch, Scotland
BRIT459	NU 9901	NW of Wooler, Scotland
BRIT348	NS 9836	W of Symington, Scotland
BRIT577	NS 3984	N of Balloch, Scotland
BRIT757	SH 9754	Llyn Brenig, Wales
BRIT389	NX 3681	Glentrool Forest, Scotland
BRIT1100	NZ 3632	N of Sedgefield, Scotland
BRIT491	NT 4054	E of Heriot, Scotland
BRIT599	NN 4888	Loch Laggan, Scotland
BRIT259	NN 6480	S of Dalwhinnie, Scotland
BRIT495	NT 4076	Port Seton, Scotland
BRIT565	NS 3212	E of Ben Vorlich, Scotland
BRIT910	NY 4010	Kirkstone Pass, England
BRIT1087	NZ 5500	Seave Green, Scotland
BRIT1122	NU 0704	N of Rothbury, Scotland
BRIT387	NX 2294	SW of Tormitchell, Scotland
BRIT866	NT 0752	Bleak Law, Scotland
BRIT191	NH 2527	Loch Beinn, Scotland
BRIT609	NT 5304	W of Wyndburgh Hill, Scotland
BRIT230	NO 0734	Gauls, Scotland
BRIT117	NO 3396	Girnock Burn, Scotland
BRIT315	NN 1880	W of Spean Bridge, Scotland
BRIT260	NN 6488	N of Dalwhinnie, Scotland
BRIT544	NS 5814	Ardchullarie More, Scotland
BRIT886	NY 4627	Dacre, England
BRIT175	NH 5740	S of An Leacainn, Scotland
BRIT99	NO 7867	SW of Johnshaven, Scotland
BRIT361	NS 8229	E of Glespin, Scotland
BRIT207	NH 1494	SE of Ullapool
BRIT274	NI 9111	E of Inverdruie, Scotland
BRIT147	NO 0861	NW of Kirkmichael, Scotland
BRIT975	NY 7607	SW of Kirkby Stephen, England
BRIT218	NH 6094	Kyle of Sutherland, Scotland
BRIT873	NY 4746	Low Heskit, England
BRIT64	NN 8260	Loch Tummel III
BRIT103	NO 8783	S of Stonehaven, Scotland
BRIT574	NS 3594	N Luss, Scotland
BRIT53	NM 4469	Sanna Bay
BRIT399	NX 5173	W of Roundfell, Scotland
BRIT504	NT 5585	North Berwick Law, Scotland
BRIT449	NT 7503	Catcleugh Reservoir, Scotland

[a] Grid coordinates are taken from British Ordnance Survey maps

Table 2.4. Most infective Continental European rust isolates.

Isolate code	Grid Co-ordinates	Origin
FRAN4	46°44'N 6°16'E	SW of Verrières, France
FRAN262	44°37'N 6°40'E	Peyre Haute, France
SVER341	56°33'N 15°48'E	Sävsjö, Sweden

Table 2.5. Additional British rust isolates employed in rust pathogenicity work.

Isolate Code	Grid Co-ordinates[a]	Origin
BRIT32	NH 8909	S of Aviemore, Scotland
BRIT38	NY 7836	Bardon Mill railway, England
BRIT51	NT 2767	Salisbury Crags II, Scotland
BRIT55	NT 2367	Craiglockhart Hill, Scotland
BRIT1144	NH 8909	S of Aviemore, Scotland
BRIT1145	NH 8909	S of Aviemore, Scotland
BRIT1146	NH 8909	S of Aviemore, Scotland
BRIT1147	NH 8909	S of Aviemore, Scotland
SELF1[b]	-	self spread onto NZ host

[a] Grid coordinates are taken from British Ordnance Survey maps

[b] This isolate had self spread on to New Zealand *H. pilosella* ex Lake Ruataniwha (source locality code = HpilLRu). The origin of the isolate was unknown but at the stage of discovery, only British rust isolates had been collected.

II.2.3 MAINTENANCE OF RUST ISOLATES

Rust isolates were maintained by re-inoculation of infected rosettes and inoculation of New Zealand *H. pilosella*. To reduce the risk of contamination of rust isolates, plants hosting an isolate were kept in separate cubicles. The cubicles were constructed of 35 cm high translucent polypropylene plastic. The infected *H. pilosella* plants were in small individual pots with the combined pot and rosette height not exceeding 10 cm.

The young leaves of already infected plants were inoculated to ensure a continuity of inoculum supply. In some cases, isolates were inoculated onto New Zealand seedlings to allow comparisons between the different isolates when the urediniospores were grown on similar hosts. Inoculations were made according to the methods of Section II.2.4.b.

In Montpellier glasshouses, the risk of rust isolate contamination was reduced by spatial separation (1.5m) of hosts infected with an isolate and rows of rust free *H. pilosella* in tall pots being placed around the infected plants. Plants were placed in

trays and watered from below, thereby minimising disturbance to the uredinia and the risk of self spread of rust in glasshouse conditions.

II.2.4 INOCULATION OF *HIERACIUM*

During the two year study period, experiments involved the inoculation of detached leaves and whole plants of New Zealand *Hieracium*. A method employing moist cotton buds to transfer urediniospores onto host surfaces was chosen to ensure an efficient use of limited supplies of inoculum.

II.2.4.a Detached leaves

Selected leaves were cut by scalpel from whole plants of the host. Leaves were placed on water agar in petri dishes covered with a lid but not sealed. A concentration of 0.95% water agar provided a soft base such that *H. pilosella* leaves could rest in full contact with the medium. Small scale experiments showed that leaf senescence of *Hieracium* spp. was sufficiently slow on water agar to not require the addition of benzimidazole, an amendment often used in detached leaf work to delay senescence. The latent period of the rust was usually less than 24 days and leaves could usually survive at least four weeks.

Occasionally leaves would sprout roots from the mid rib of the leaf base and in some cases organogenesis would eventually occur, with a rosette forming from the leaf base. In most cases, however, leaves did not form roots or rosettes.

Rust urediniospores were normally collected from uredinia by brushing several infected leaf surfaces with a slightly moist cotton bud. Inoculation was carried out by further moistening the cotton bud with a drop of distilled water and brushing the leaf surfaces, leaving moisture and urediniospores. This resulted in several hundred urediniospores being deposited per cm^2 of leaf surface. Eye and hand lens observations ensured as even a spread of urediniospores on the leaf surface and as equal amounts of spores per leaf as practicable. Methods of inoculation which are

more reliable in giving an even spread of urediniospores, e.g., spraying a spore suspension, were usually not practicable because of the small amounts of inoculum of each strain available at one time.

In Edinburgh, petri dishes were kept in darkness for 16h (ca. 15°C) after inoculation, during which time germ tubes would be produced and extend. Subsequently, the petri dishes were placed in the light, either in the laboratory (ca. 17°C daytime, 13°C night; without night time light) or glasshouse (13°C daytime min., 10°C night-time min.; without artificial light). In Montpellier, petri dishes were kept in sealed moist chambers for 16h (ca. 20°C; 8h dark then 8h light). Covers were then taken off the chambers and petri dishes were kept in the moist chambers (ca. 20°C; 16h light / 8h dark) for four weeks.

Detached leaves were monitored at least twice weekly over four weeks for symptoms and the production of sori was recorded.

II.2.4.b Whole plants

Urediniospores were collected with slightly moistened cotton buds by brushing the surfaces of several infected leaves. As for detached leaves, inoculation was carried out by moistening the cotton bud with a drop of distilled water and brushing the leaf surfaces, leaving moisture and urediniospores. Inoculation was more or less even over the entire plant. In Edinburgh, inoculated plants were placed in plant trays with clear plastic covers. Plants and the inside of the covers were sprayed with distilled water to provide a humid environment. The plants were kept in glasshouse conditions (ca. 13°C day time min., 10°C night time min.; natural light/darkness). Plants remained covered for 16 h and the covers were then removed. Each leaf was examined for symptoms and the production of sori for up to six weeks, post-inoculation.

In Montpellier, inoculated plants were placed in covered moist chambers (ca. 20°C; 8h dark, 8h light). Plants and the sides of the chamber were sprayed with distilled water.

After 16h the covers were taken off the chambers and plants were left in the chambers for ca. 8h to prevent too rapid drying, before the plants were transferred to the glasshouse (ca. 20°C max., 16°C min.; natural light or ca. 22°C, 18°C min with intermittent fogging; natural light).

The propagation of New Zealand *Hieracium* plants from seed is detailed in Section II.3.1.a.

II.3 HOST COLLECTIONS

Representatives of a number of *Hieracium* taxa, particularly *H. pilosella*, were collected for pathogenicity work. Collections were made in New Zealand and overseas.

II.3.1 METHODS OF COLLECTION

II.3.1.a New Zealand *Hieracium*

Hieracium taxa were collected from a range of high country sites, over two seasons,. In the first season, 22 collections were made, including one site of *H. aurantiacum*, one site of *H. caespitosum*, one site of *H. lepidulum*, 11 sites of *H. pilosella*, seven sites of *H. praealtum*, and two sites of *H.* x *stoloniflorum*. In the second season, six further sites of *H. caespitosum*, 15 of *H. pilosella*, 11 of *H. praealtum*, and three of *H.* x *stoloniflorum* were collected.

At each site, seed was taken from an area of as great as 0.25 hectares, with entire capitula occasionally being included in the collections. Seed was stored in paper envelopes at room temperature before and after transport to the United Kingdom for experimental work.

Propagation in Edinburgh was by one of two methods. One method was sowing a number of achenes in a small pot of seed raising mix (1 part John Innes No. 1 seed

raising mix : 1 part coarse sand). Achenes germinated best when planted very shallow. The rate of germination, and seedling emergence and growth, varied greatly between individuals. Small seedlings were transplanted to small (7 cm by 7 cm by 8 cm deep) individual pots when their rosettes were over 1 cm in diameter. Seedlings raised for experimentation in Edinburgh were propagated by placing achenes on water agar (0.95% agar, no amendment) in closed petri dishes. The water agar method ensured a high percentage of germination over a short period of time. Seedlings were transplanted from water agar after the emergence of the first true leaf, into small individual pots (1 part John Innes No.2 potting mix : 1 part peat).

Plants were watered either regularly from above or from below with shallow trays of water. In the United Kingdom, plant quarantine regulations prohibited any of the plants raised from New Zealand seed being allowed to set new seed, or to be planted outside of glasshouses.

Seed sowing in Montpellier was by the water agar method as used in Edinburgh. Seedlings were transplanted at the first true leaf stage, into small individual pots (2 parts soil : 1 part coarse sand : 1 part peat).

II.3.1.b European *Hieracium*

Plants of European *Hieracium* were transplanted from the field or private gardens to individual pots in the glasshouse. Plants were generally only collected if they were host to rust infection, a few collections were also made of species of particular interest or for testing susceptibility to rust infection.

Most collections of European plants were made during extensive field surveys. These plants were treated according to the methods in Section II.3.2 below.

II.3.2 NEW ZEALAND *HIERACIUM* COLLECTIONS.

A code was given to each locality of *Hieracium* collections (Table 2.6 for *H. pilosella* and Table 2.7 for other *Hieracium* species; listed by latitude) and these codes are used throughout the thesis. Subspecies identifications, grid reference and the date of seed collection were recorded for each. Subspecies identification in *H. pilosella* was based on hairs of the involucral bracts, according to the descriptions in Sell and West (1976). Although most of the specimens were identified as subspecies *micradenium*, some of these contained a very small degree of simple eglandular hairs on the bracts which approached the description of the other two subspecies recorded in New Zealand by Garnock-Jones (in Webb *et al.*, 1988). Specimens were only identified as subspecies *pilosella* and *trichosoma* if the numbers of simple eglandular hairs on the bracts were more than a few. Subspecies *micradenium* was reported to be the most common of the subspecies in New Zealand (Garnock-Jones in Webb *et al.*, 1988). Voucher specimens of adult plants, involucre and seed of 19 of these collections are lodged in the Manaaki Whenua/Landcare Research Herbarium at Lincoln (CHR 468901 to 468920).

Table 2.6. Source localities and coding of New Zealand *H. pilosella* collections.

TAXA[a]	CODE	ORIGIN OF SEED	GRID REFERENCE
H. pilosella (m)	HpilDRd	Desert Rd (North Island)	39°09.8'S 175°45.9'E
H. pilosella (m)	HpilTUa	NW of Tuamarina	41°24.9'S 173°55.5'E
H. pilosella (m)	HpilLVa	Lee Valley	41°26.6'S 173°09.0'E
H. pilosella (m)	HpilHSp	Hanmer Springs	42°27.4'S 172°54.4'E
H. pilosella (m)	HpilJPa	Jollies Pass	42°27.4'S 172°54.5'E
H. pilosella (m)	HpilHBp	Banks Peninsula	43°49.2'S 173°00.7'E
H. pilosella (m,t)	HpilCRa1	Craigieburn (I)	43°09.6'S 171°44.3'E
H. pilosella (m)	HpilCRa2	Craigieburn (II)	43°09.6'S 171°44.3'E
H. pilosella (m)	HpilLLy	Lake Lyndon	43°17.8'S 171°42.5'E
H. pilosella (m)	HpilLCo	Lake Coleridge	43°20.4'S 171°37.4'E
H. pilosella (m)	HpilLRd	Lyndon / Coleridge Rd	43°20.5'S 171°38.2'E
H. pilosella (m,p,t)	HpilLCl	Lake Clearwater	43°37.2'S 171°03.6'E
H. pilosella (m)	HpilHEr	Mt. Cook Hermitage	43°44.0'S 170°06.6'E
H. pilosella (m)	HpilGPs	Godley Peaks	43°51.9'S 170°28.3'E
H. pilosella (m)	HpilMJb	Mt. John base	43°59.1'S 170°27.7'E
H. pilosella (m)	HpilMJt	Mt John trial site[b]	43°59.1'S 170°27.7'E
H. pilosella (m)	HpilMJw	W face of Mt. John	43°59.1'S 170°27.8'E
H. pilosella (m)	HpilLTe	Lake Tekapo	44°00.5'S 170°28.8'E
H. pilosella (m)	HpilHOs	Holbrook Station	44°05.2'S 170°31.9'E
H. pilosella	HpilDKc	Dog Kennel Corner	44°05.8'S 170°34.1'E
H. pilosella (m)	HpilTWo	The Wolds	44°06.0'S 170°21.4'E
H. pilosella (m)	HpilGEr	Geraldine	44°07.1'S 171°12.5'E
H. pilosella (m)	HpilMAs2	Maryburn Station (II)	44°11.1'S 170°19.0'E
H. pilosella (m)	HpilMAs1	Maryburn Station (I)	44°11.1'S 170°27.7'E
H. pilosella (m)	HpilLRu	Lake Ruataniwha	44°15.9'S 170°04.4'E
H. pilosella (m)	HpilTWz	Twizel	44°16.2'S 170°04.0'E
H. pilosella (m)	HpilCAv	Cave (Church)	44°19.2'S 170°56.3'E
H. pilosella	HpilODs	Ohau Downs Station	44°19.8'S 169°57.7'E
H. pilosella (m)	HpilHAs	Haldon Station	44°21.0'S 170°17.0'E
H. pilosella (m)	HpilSCs2	Stony Creek Station (II)	44°21.3'S 170°23.9'E
H. pilosella (m)	HpilCAn	Cannington	44°22.1'S 170°56.2'E
H. pilosella (m)	HpilCVa	Craigmore Valley	44°26.0'S 171°00.2'E
H. pilosella (m)	HpilSCs1	Stony Creek Station (I)	44°26.8'S 170°34.1'E
H. pilosella	HpilTHs	Tara Hill Station	44°32.2'S 169°54.3'E

[a] The letters in brackets indicate that some specimens matched subspecies described in the Flora Europaea by Sell and West (1976).

(m)=subsp. *micradenium*
(p)=subsp. *pilosella*
(t)=subsp. *trichosoma*.

[b] The Mt. John trial site is a high country agricultural research site administered by the New Zealand Pastoral Agriculture Research Institute Ltd. (AgResearch).

Table 2.7. Source localities and coding of New Zealand *Hieracium* spp. collections.

TAXA[a]	CODE	ORIGIN OF SEED	GRID REFERENCE
H. aurantiacum (c)	HaurPPa	Porter's Pass	43°17.6'S 171°44.2'E
H. caespitosum (c)	HcaeLCl	Lake Clearwater	43°37.2'S 171°03.6'E
H. caespitosum (c)	HcaeMJt	Mt. John trial site	43°59.1'S 170°27.7'E
H. caespitosum (c)	HcaeDKc	Dog Kennel Corner	44°05.8'S 170°34.1'E
H. caespitosum (c)	HcaeMJw	W face of Mt John	43°59.1'S 170°27.8'E
H. caespitosum (c)	HcaeLRd	Lyndon / Coleridge Rd	43°20.5'S 171°38.2'E
H. caespitosum (c)	HcaeLTe	Lake Tekapo	44°00.5'S 170°28.8'E
H. caespitosum (c)	HcaeMAs	Maryburn	44°11.1'S 170°27.7'E
H. lepidulum	HlepLRd	Craigieburn	43°20.4'S 171°38.1'E
H. praealtum (t)	HpraCRa	Craigieburn	43°10.2'S 171°44.3'E
H. praealtum (p)	HpraLCl	Lake Clearwater	43°37.2'S 171°03.6'E
H. praealtum (t)	HpraMJt	Mt. John trial site	43°59.1'S 170°27.7'E
H. praealtum (t)	HpraMJs	SW face of Mt. John	43°59.6'S 170°27.2'E
H. praealtum (t)	HpraTWo	The Wolds	44°06.0'S 170°21.4'E
H. praealtum (t)	HpraLRu	Lake Ruataniwha	44°15.9'S 170°04.4'E
H. x *stoloniflorum*	HstoCRa	Craigieburn	43°09.6'S 171°44.3'E
H. x *stoloniflorum*	HstoMJs	SW face of Mt. John	43°59.6'S 170°27.2'E
H. x *stoloniflorum*	HstoLTe	Lake Tekapo	44°00.5'S 170°28.8'E

[a] The letters in brackets indicate that some specimens matched subspecies described in the Flora Europaea by Sell and West (1976).

H. aurantiacum : (c) = subsp. *carpathicola.*
H. caespitosum : (c) = subsp. *caespitosum.*
H. praealtum : (p) = subsp. *praealtum.*
(t) = subsp. *thaumasium.*

II.3.3 NON NEW ZEALAND *HIERACIUM* USED AS HOSTS IN RUST EXPERIMENTS.

Several collections of *H. pilosella* from Scotland, Sweden and Chile and one collection of *H.* x *stoloniflorum* were tested for susceptibility to rust isolates. One aim was to assess the host range of rust isolates selected for New Zealand *H. pilosella* biological control. Another aim was to assess the extent to which host genetic variation affected the distribution of rust within Scottish populations. The localities of the host collections were recorded (Table 2.8).

Table 2.8. Source localities and coding of non New Zealand *Hieracium* plants used as hosts in rust experiments.

Species	Code	Origin	Grid Reference [a]	Date Collected
H. pilosella	Bush	Bush Estate, Mid Lothian, UK	NT 2563	July, 1993
	Sävsjö[b]	Sävsjö, Sweden	56°33'N 15°48'E	May, 1993
	Chile	Southern Chile, South America	-	Jan, 1993
	Alcove	near Aviemore, UK	NH 8808	July, 1993
	Nursery (iii-v)	near Aviemore, UK	NH 8807	July, 1993
	Polchar	near Aviemore, UK	NH 8910	July, 1993
	Woodpecker Gully (i)	Holyrood Park, Edinburgh, UK	NT 2873	July, 1993
	Woodpecker Gully (ii)	Holyrood Park, Edinburgh, UK	NT 2873	July, 1993
	Samson's Ribs (xii)	Holyrood Park, Edinburgh, UK	NT 2873	July, 1993
	Cat Nick Slope (i)	Holyrood Park, Edinburgh, UK	NT 2873	July, 1993
	South Quarry A (viii)	Holyrood Park, Edinburgh, UK	NT 2873	July, 1993
	South Quarry A (ix)	Holyrood Park, Edinburgh, UK	NT 2873	July, 1993
	Haggis Knowe (i)	Holyrood Park, Edinburgh, UK	NT 2874	July, 1993
	Haggis Knowe (ii)	Holyrood Park, Edinburgh, UK	NT 2874	July, 1993
	Haggis Knowe (iii)	Holyrood Park, Edinburgh, UK	NT 2874	July, 1993
	Haggis Knowe (iv)	Holyrood Park, Edinburgh, UK	NT 2874	July, 1993
	Haggis Knowe (v)	Holyrood Park, Edinburgh, UK	NT 2874	July, 1993
	Haggis Knowe (vi)	Holyrood Park, Edinburgh, UK	NT 2874	July, 1993
H. x *stoloniflorum*	Watling Garden	Private Garden, Edinburgh, UK	NT 2185	Sept, 1992

[a] Grid References for UK isolates taken from British Ordnance Survey maps.

[b] The Sävsjö host plants were infected with rust (isolate SVER341, see Section II.2.2) when collected from the field.

Chapter III: HOST / PATHOGEN RELATIONSHIPS OF RUST DISEASE - LABORATORY AND GLASSHOUSE STUDIES

III.1 INFECTION PROCESS

III.1.1 INTRODUCTION

The infection process of *P. hieracii* var. *piloselloidarum* following urediniospore deposition on a host surface was investigated. Observations of the infection process were made with a dissecting microscope, fluorescent light microscope and scanning electron microscope (SEM).

III.1.2 MATERIALS AND METHODS

Observations were made of all the stages of the urediniospore infection process. Both whole rosettes and detached leaves were inoculated with urediniospores for the purposes of these observations. Inoculation methods were as described in Section II.2.4.a and II.2.4.b. All inoculated hosts were *H. pilosella* grown from seed collected from New Zealand localities. Dissecting microscope investigations were made with a camera fitted to the microscope. The methods for clearing and fluorescent staining of leaves and for scanning electron microscopy are given below.

A leaf clearing and fluorescent staining technique, adapted by Helfer (1986) from Rohringer *et al.* (1977), was used on 10 mm by 10 mm sections of host leaf (for Figs 3.6-3.12). The leaf sections were transferred to lactophenol/ethanol (1:2); fixed by boiling for 90s (ca. 81°C); left for 16h overnight; washed twice for 15 min each in 50% v/v ethanol; washed twice for 15 min each in 0.05N NaOH; washed in distilled water three times for 10 min each; stained by transferring to Calcofluor White M2R New (Cyanamide) 0.1% in 0.1M Tris HCl buffer pH 8.5 for 5 min.; washed in distilled water four times for 10 min each; transferred to glycerol (25%) for 30 min; mounted in glycerol (50%) with 0.1% lactophenol for preservation. Slides were examined using an Olympus BH2 microscope fitted with epifluorescence equipment

using an Ushio USH-102D 100W high pressure mercury burner and UG1 exciter filters at 495 + 545 nm and barrier filters at 515 + 590 nm.

Scanning electron microscope specimens were prepared by using one of three methods. The first method (Figs 3.7 and 3.10) involved freeze drying the specimen; splutter coating with platinum; and examination under a Cambridge Stereoscan 250 SEM at 10 kV. The second method (Figs 3.8 and 3.9) involved freeze drying then freeze fracturing of specimens and examination without coating under cold stage electron microscopy with a Zeiss Digital Electron Microscope X2000 SEM at 5 kV. The third method (Fig 3.11) also involved examination under the Zeiss SEM at 5 kV; specimens were freeze dried and coated with platinum.

III.1.3 RESULTS AND DISCUSSION

The predominant life cycle stage noted in the field and glasshouse was uredinia producing asexual urediniospores. Urediniospores were the inoculum used in most of the studies of this thesis and in the work of Scott (pers. comm. 1995a). The urediniospores are echinulate and globoid to ellipsoid (see Fig 3.10). There are two slightly supra-equatorial germ pores on each urediniospore.

The infection process is shown in Figs 3.1-3.12. Germination of the urediniospore involves the production of one or sometimes two germ tubes (Figs 3.1 and 3.7). Germ tubes form an appresorium over a host stoma to secure penetration of the pore by a rust hypha leading to the formation of a substomatal vesicle. Mycelium arising from the vesicle grows intercellularly in the host tissue. Haustorial mother cells (Fig 3.8) on the mycelium give rise to haustoria which penetrate host cell walls and obtain nutrients. The rust mycelium continues to grow to around a 1 mm radius from the point of penetration (Figs 3.2, 3.3 and 3.4). Fig 3.9 shows rust mycelium growing intercellularly in leaf tissue. A new uredinium is formed from a concentration of hyphae below the host epidermis (Fig 3.3; Fig 3.9 bottom left) and the uredinium produces urediniospores that eventually rupture the host epidermis and cuticle (Figs 3.4, 3.5, 3.12).

The period of time between inoculation and eruption of the uredinium is termed the latent period. The latent period was variable, with the shortest recorded period being seven days with isolate ÉIRE14 on detached leaves (ca. 18°C) and the longest being a possible 40 day period on a whole rosette inoculated with isolate BRIT32 (ca. 13°C). Latent periods of rusts frequently vary by these magnitudes, for example the latent period of the groundnut rust *Puccinia arachidis* was shown by Wadia and Butler (1994), to vary from 12 to 49 days dependant on temperature, with longer periods at lower temperatures.

The infection from one urediniospore was localised, with usually just one sorus being produced. Limited spread of rust hyphae in the host tissue occasionally produced compound sori (Figs 3.6 and 3.11). Infrequently there was a shotholing effect where a ring of sori resulted in plant tissue within the ring going necrotic and falling off the leaf (Fig 3.13). Uredinia were occasionally amphigenous (sori arising from one infection focus erupting on both surfaces of a leaf), more so in severe infections. With *Puccinia hieracii* var. *hieracii* rust infections on thinner leaved species such as *Taraxacum officinale*, often the majority of sori are amphigenous (pers. obs.).

Ayres and Paul (1986) pointed out that the sizes of rust sori are enormous compared to a fully open stoma, leading to increased environmental stress on the host (see Section I.4.7). The relative sizes of an *Hieracium* rust sorus and a stoma can be seen in Figs 3.10 and 3.11. Rust infection was sometimes observed to hasten leaf senescence (Fig 3.16).

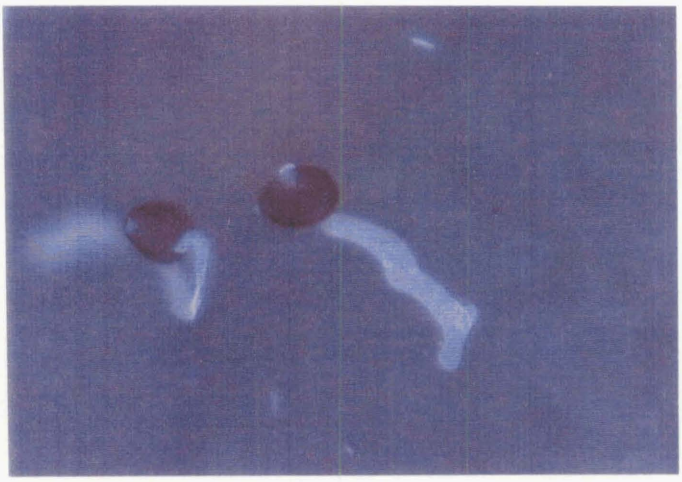
An *H. pilosella* ramet (rosette produced on a stolon) is shown with a severe rust infection (Fig 3.14). Fig 3.15 shows a New Zealand *H. pilosella* infected with a rust with chlorosis evident around the uredinia, particularly on older leaves. A severe infection by isolate BRIT53 on *H. pilosella* from New Zealand was characterised by a silver colouration caused by spores underneath the raised epidermis.

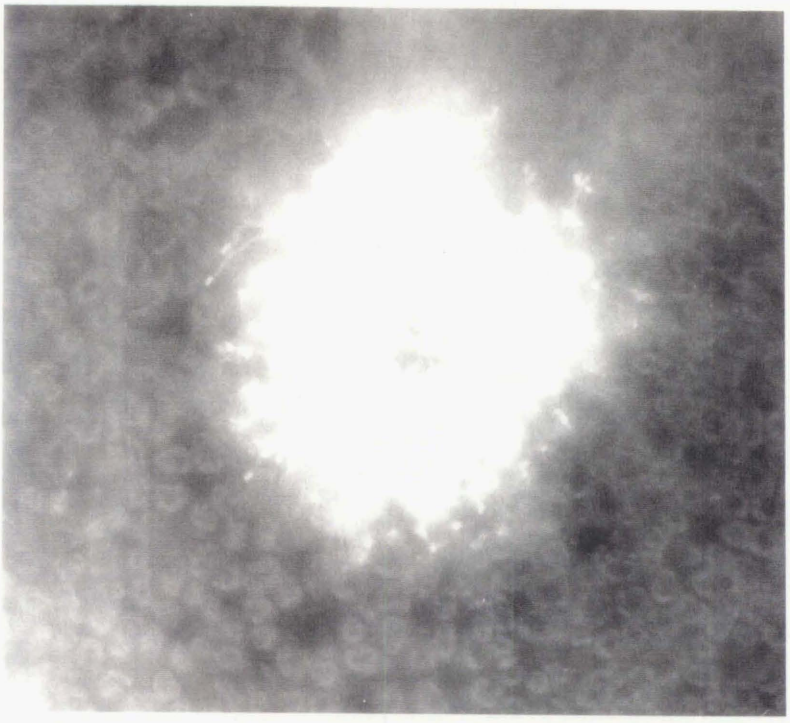
Rust was observed to overwinter as uredinia on *H. pilosella* (Fig 3.18) though the proportions of sori surviving winter were low. The sexual phases of the life cycle were not commonly observed in the Edinburgh area and may not play a large role in the survival of rust over winter there.

Fig 3.1 **Germinating urediniospores**
FLUORESCENT LEAF CLEARING (x 200)
Germinating urediniospores on a *H. pilosella* leaf surface. The germ tubes have taken up fluorescent stain.
|————————|
50μ m

Fig 3.2 **Intercellular growth of rust in host tissue**
FLUORESCENT LEAF CLEARING (x 100)
After penetration, rust mycelium expands between *H. pilosella* leaf cells. The fluorescent staining is taken up by fungal cells while host cells remain dark.
|————————|
100μm

Fig 3.3 **Formation of uredinium**
FLUORESCENT LEAF CLEARING (x 100)
Following intercellular expansion of rust mycelium, a uredinium is formed amongst a concentration of hyphae. Immature urediniospores can be seen in the centre of the brightly stained mycelium.
|————————|
100μm





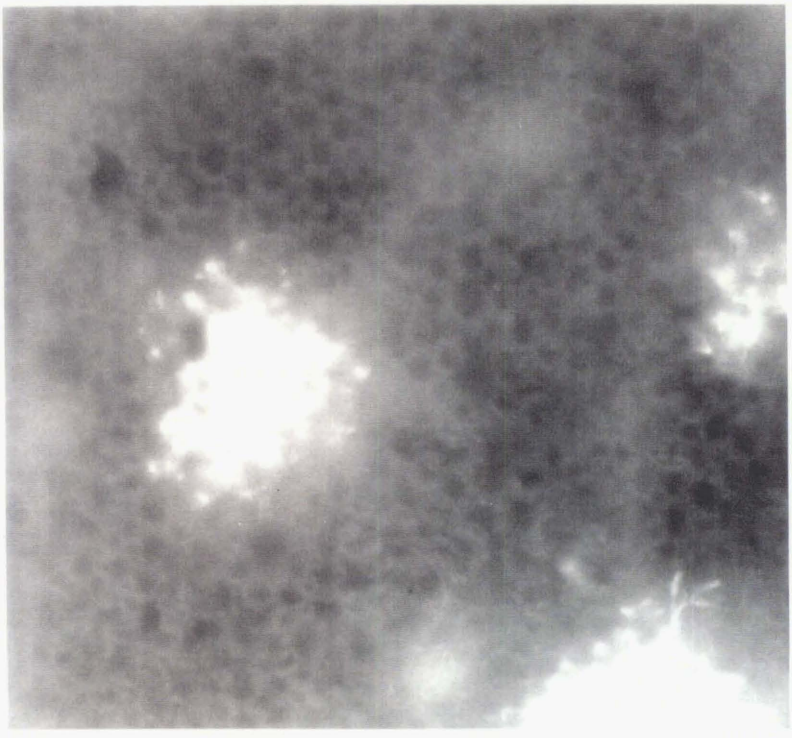
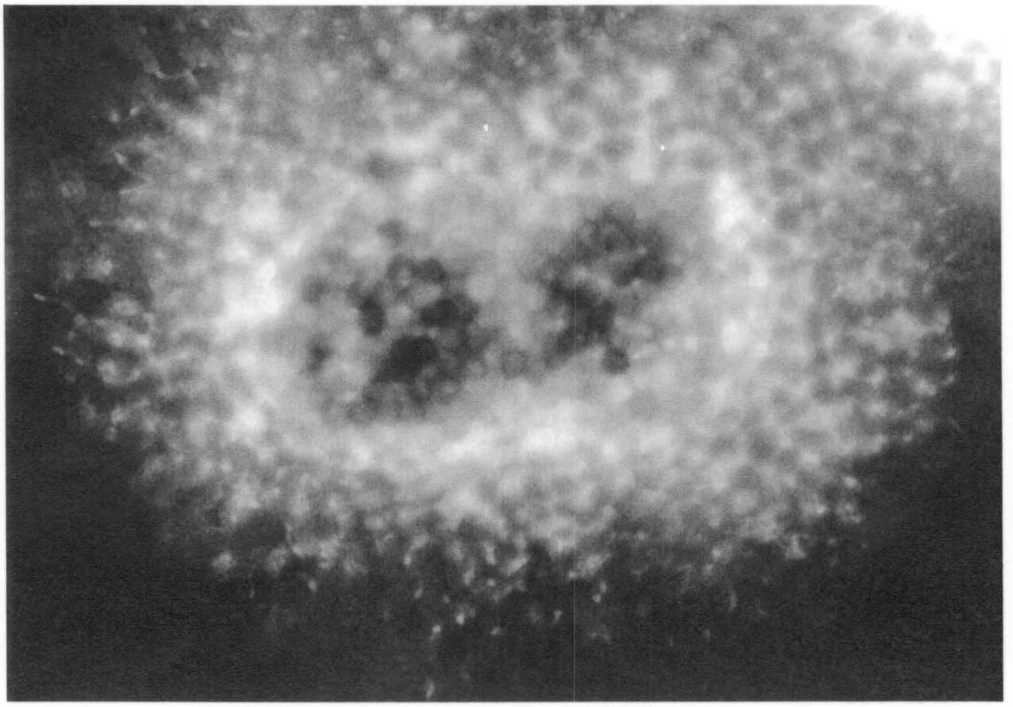


Fig 3.4 **Uredinium prior to rupturing the leaf surface**
FLUORESCENT LEAF CLEARING (x 100)
Rust uredinium still beneath an intact epidermis and cuticle. Immature spores take up some fluorescent stain, while the more mature spores remain darker.
|————————|
100μm

Fig 3.5 **Uredinium after rupturing the leaf surface**
FLUORESCENT LEAF CLEARING (x 100)
An erupting uredinium. The pressure from continual production of urediniospores ruptures the *H. pilosella* epidermis and cuticle, exposing the spores.
|————————|
100μm



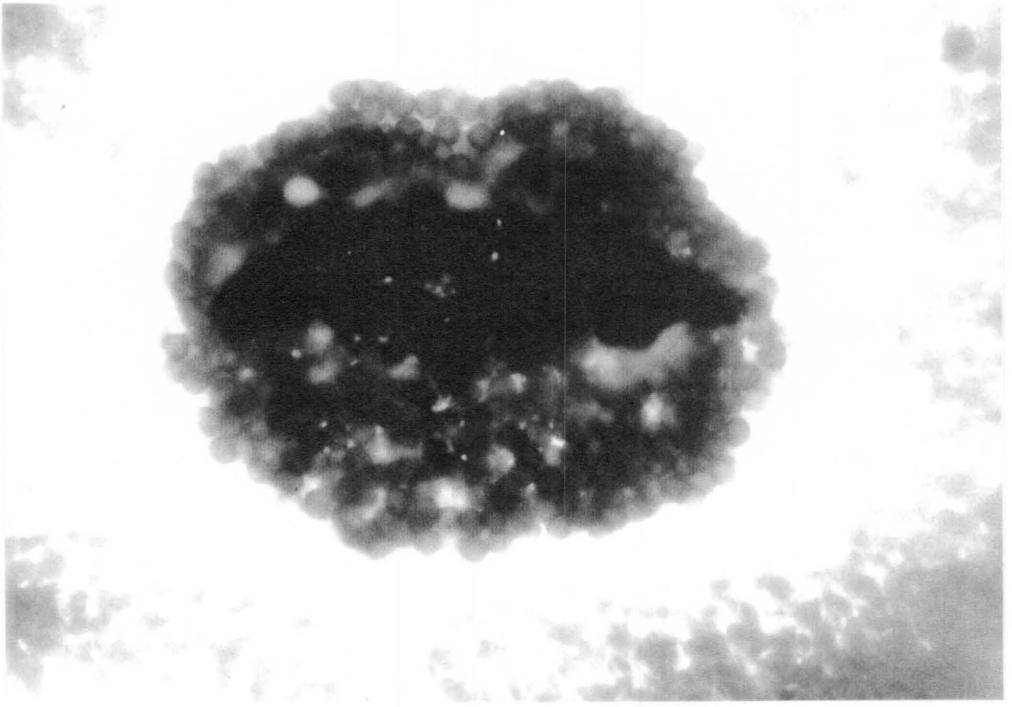
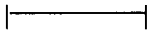


Fig 3.6 **Compound uredinium**

FLUORESCENT LEAF CLEARING (x 100)

Note the three areas where urediniospores are being produced in between the mass of brightly stained rust mycelium. A germinating urediniospore can be seen at the bottom right of the picture.



100μm

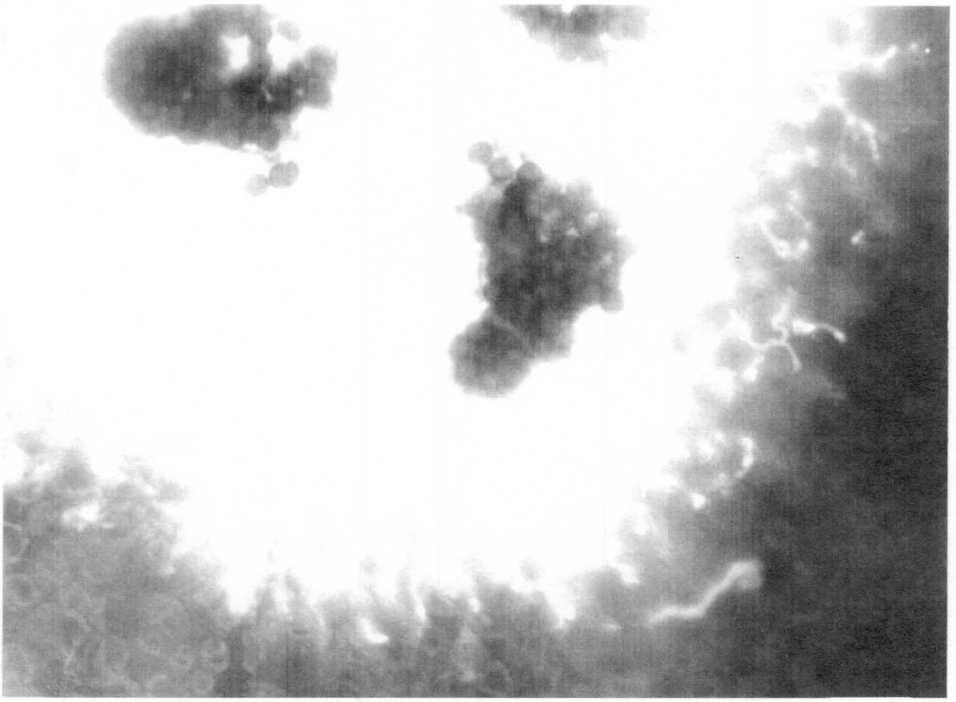


Fig 3.7 **Urediniospore germination**

SCANNING ELECTRON MICROGRAPH (x 340)

Urediniospores deposited near a sorus. One urediniospore has germinated, producing a single germ tube.

|——————|

50μm

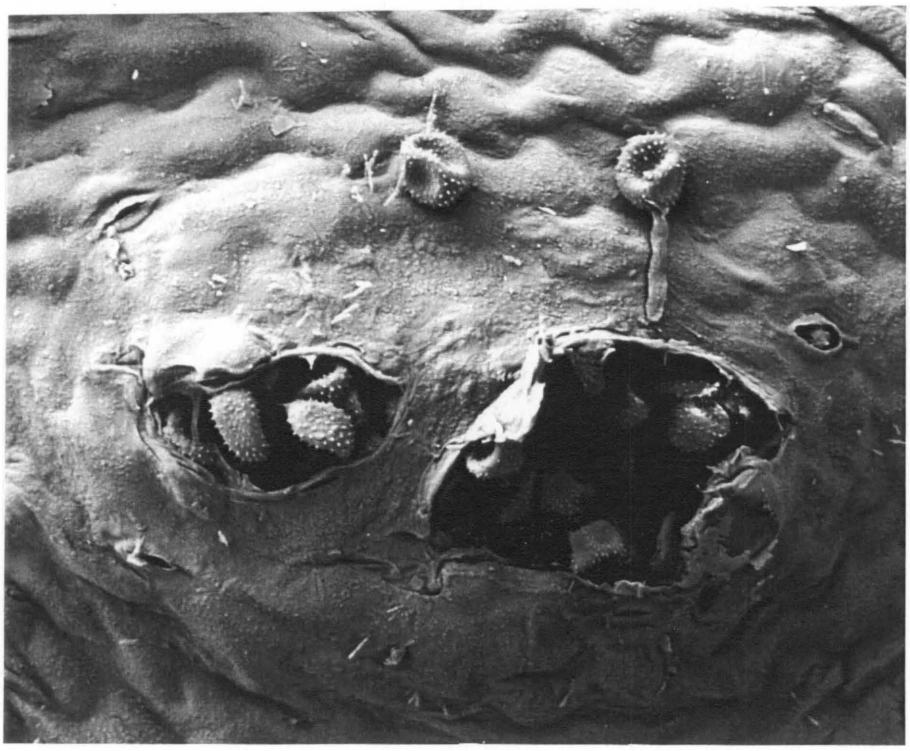
Fig 3.8 **An haustorial mother cell of rust**

SCANNING ELECTRON MICROGRAPH (FREEZE FRACTURE) (x5000)

Mother cell arises from a rust hypha, which would produce an haustorium, penetrating the host cell wall and obtaining resources.

|——————————|

5μm



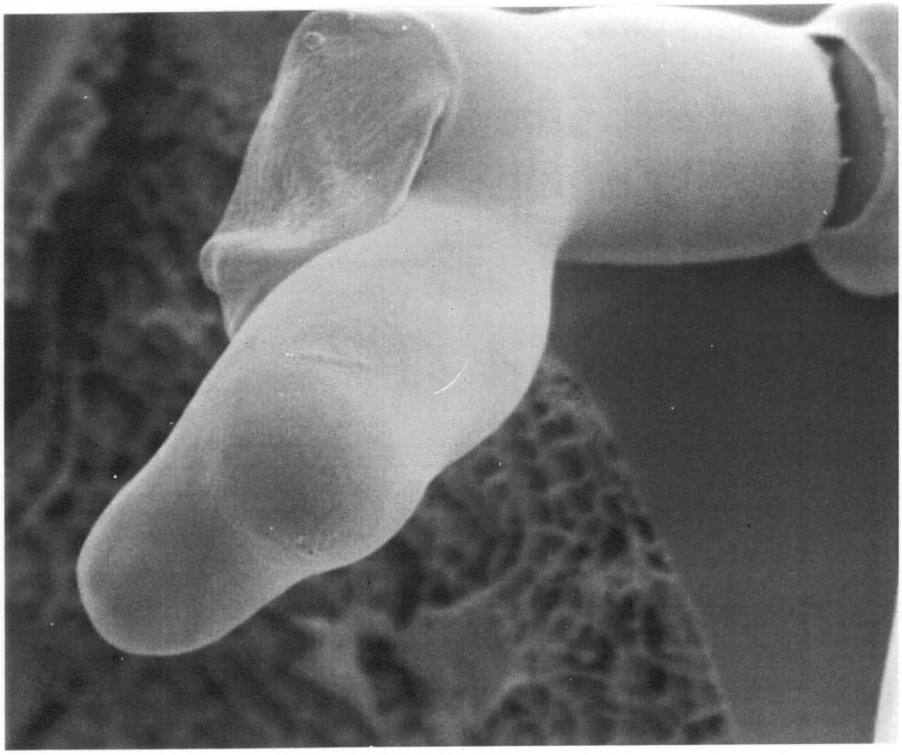


Fig 3.9 **Intercellular rust mycelium**

SCANNING ELECTRON MICROGRAPH (FREEZE FRACTURE) (x 500)
Transverse section of a *H. pilosella* leaf. Rust hyphae grow in amongst the host cells. At the bottom left of the micrograph, rust mycelium is concentrated, beginning to form a uredinium.

50μm

Fig 3.10 **Uredinium with urediniospores**

SCANNING ELECTRON MICROGRAPH (x 420)
Urediniospores exposed for dispersal. Note the relative sizes of a stoma (right hand side) and the rupture in host tissue caused by a uredinium.

50μm

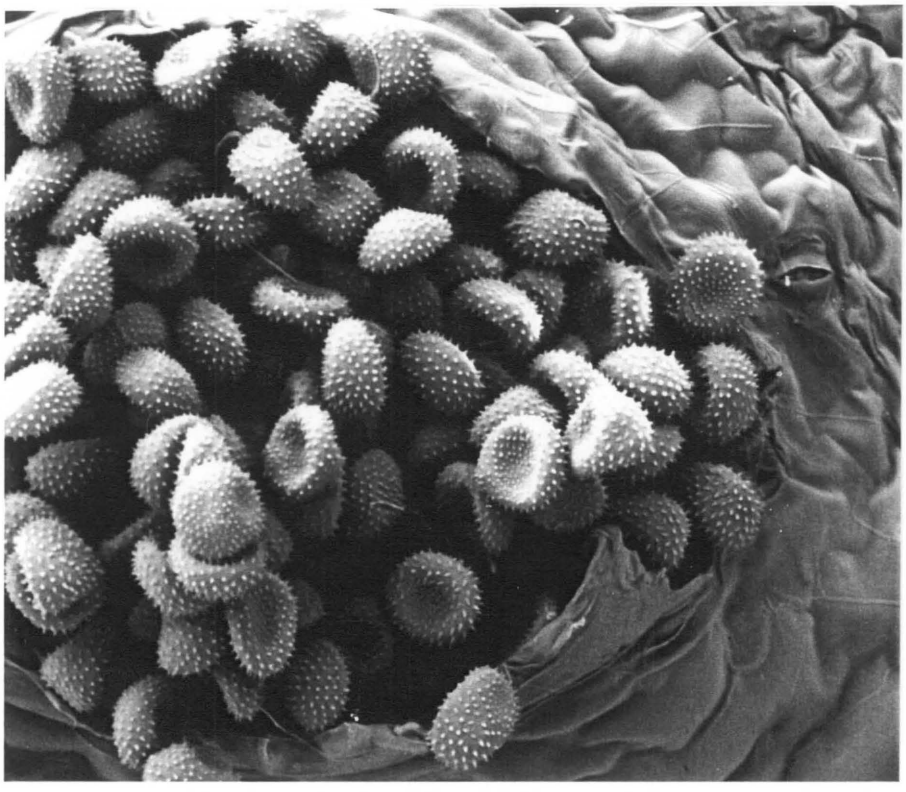


Fig 3.11 **Compound uredinium**

SCANNING ELECTRON MICROGRAPH (x100)

A large compound uredinium rupturing an *H. pilosella* leaf surface.

200μm

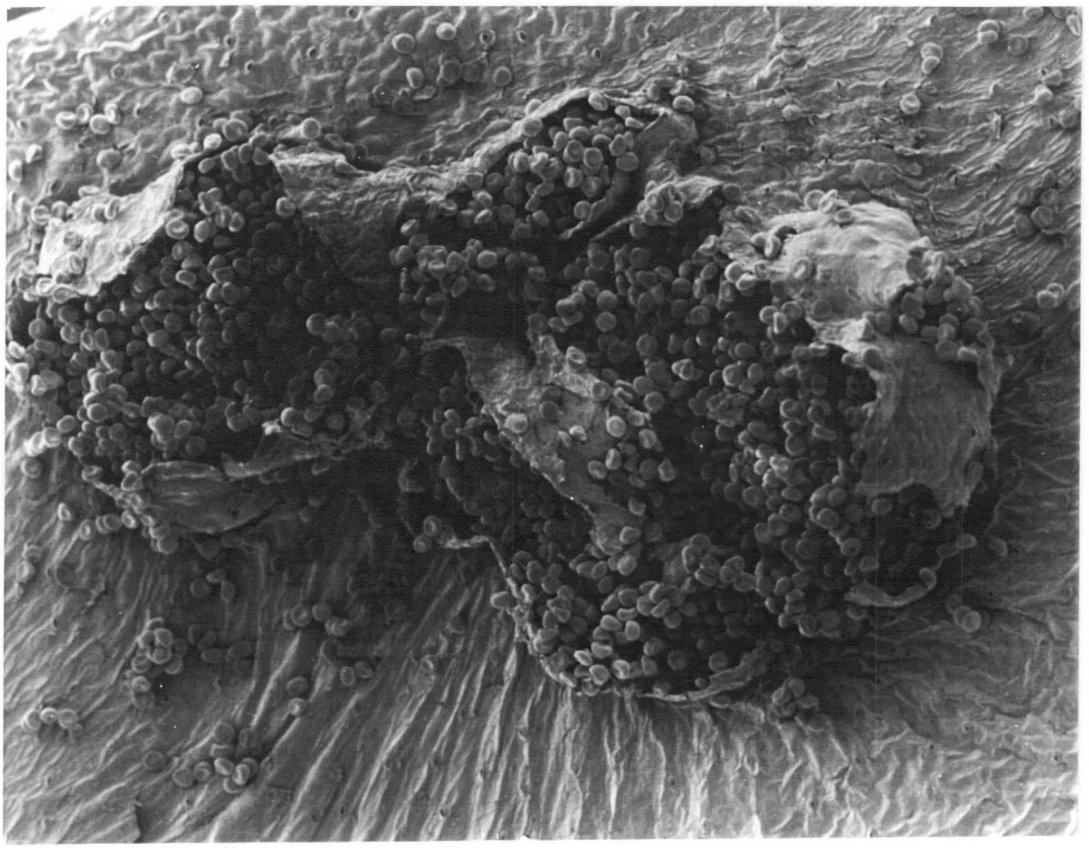
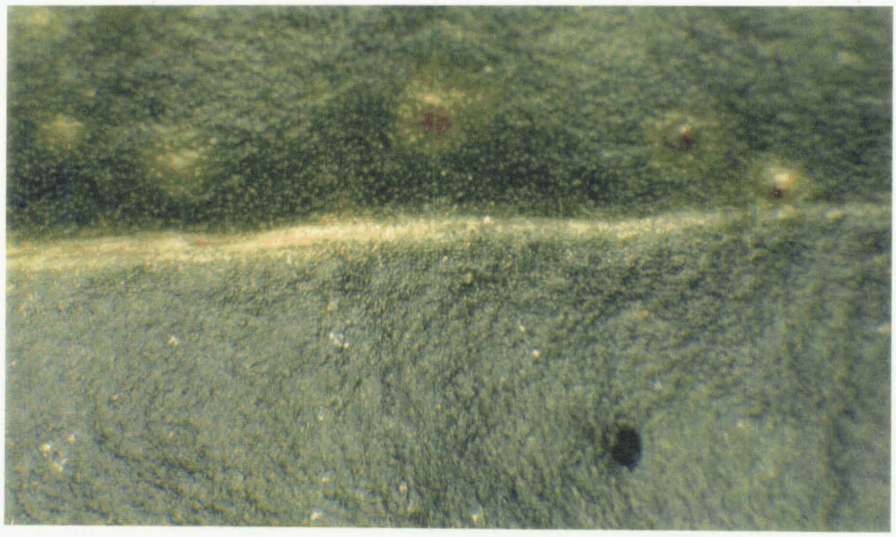


Fig 3.12 **Uredinia forming**
MACROSCOPE (x 10)
Note a slight yellowing and raising of the area of a sorus, prior to eruption.

Fig 3.13 **Shot-holing effect of rust infection**
MACROSCOPE (x 40)
Shot-holing effect. A ring of rust uredinia caused the centre of host (*H. pilosella*) leaf tissue to become necrotic and fall out.

Fig 3.14 **A severe rust infection on a young *H. pilosella* ramet**
Note the infection on both upper (adaxial) and lower (abaxial) surfaces of the leaf.



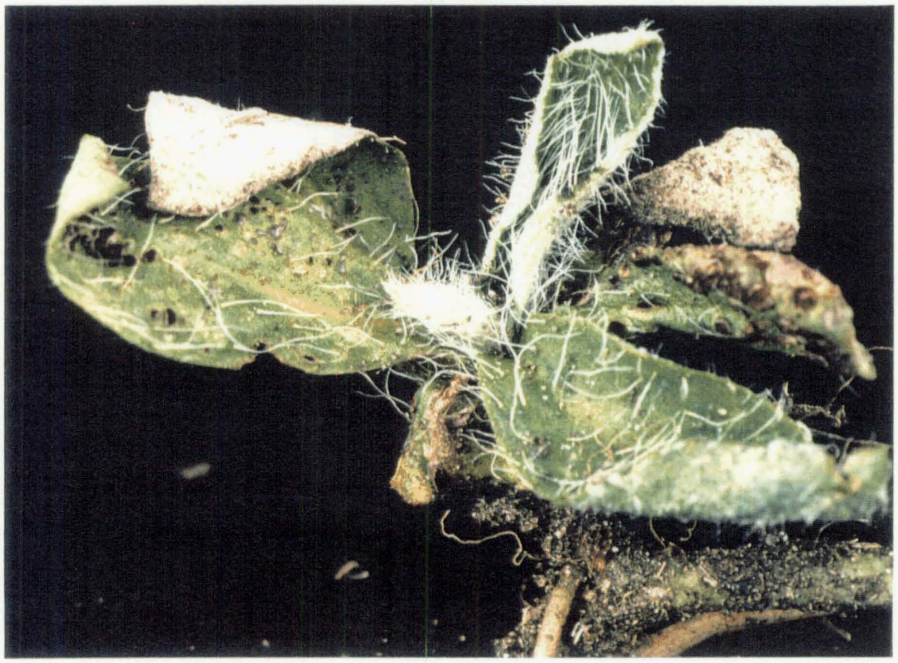


Fig 3.15 **Moderate rust infection**
Isolate BRIT32 on *H. pilosella* ex Haldon Station, New Zealand (HpilHAS). Note chlorosis around uredinia on some older leaves.

Fig 3.16 **Leaf senescence**
Senescence of an old leaf hastened by rust infection. Note the necrotic tissue surrounding many of the uredinia.

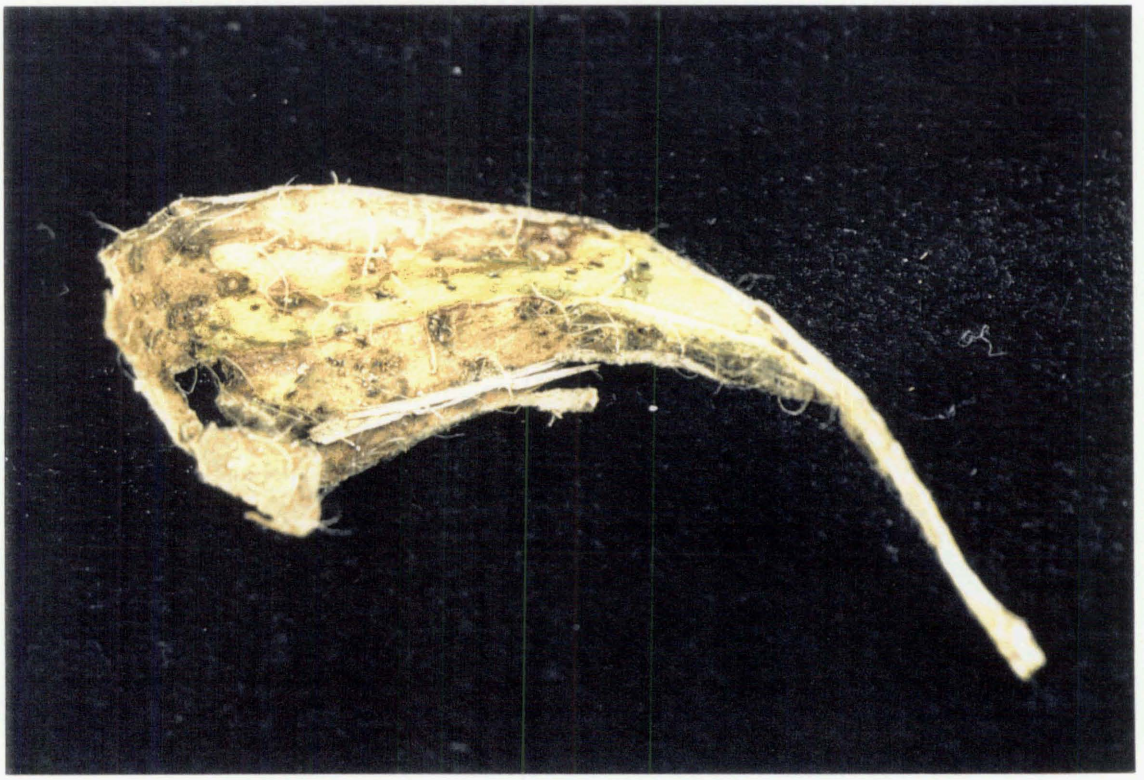
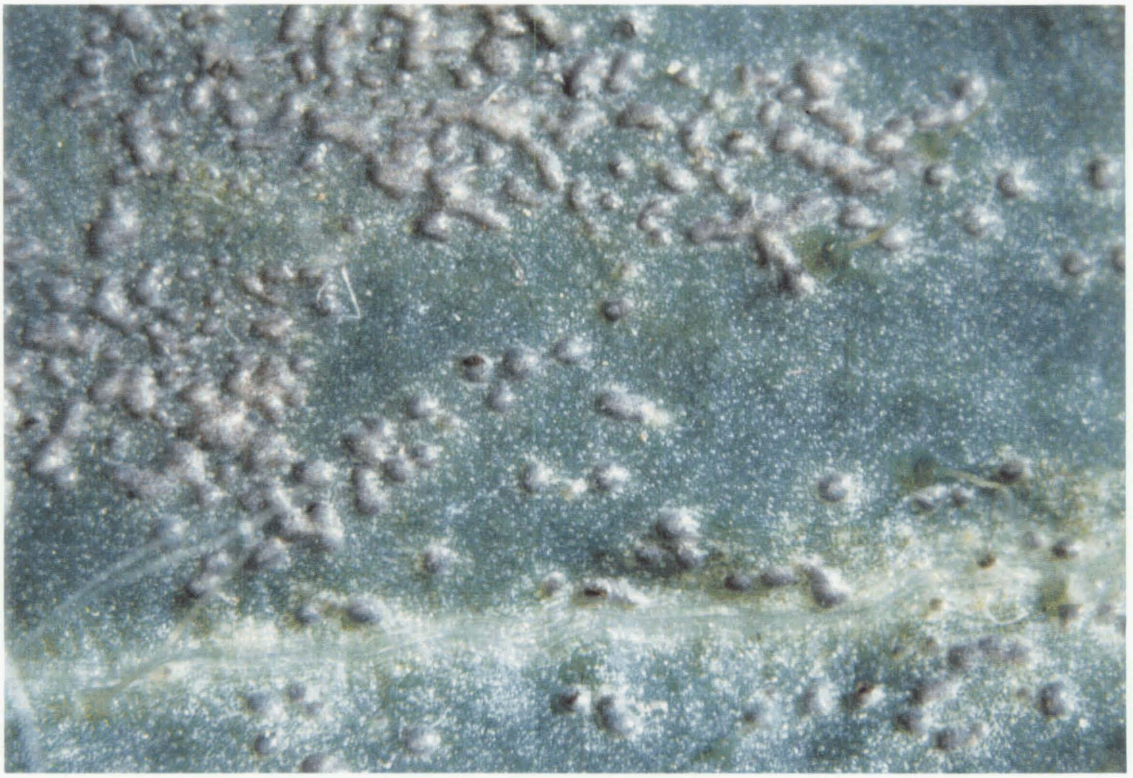


Fig 3.17 **Severe rust infection**
MACROSCOPE (x 40)
Isolate BRIT53 on *H. pilosella* ex Lake Coleridge, New Zealand (HpilLCo).
Most uredinia have yet to erupt.

Fig 3.18 **Over wintering rust**
On *H. pilosella*, Holyrood Park, Edinburgh (December 1993). Note that many rust sori are surrounded by necrotic lesions.



III.2 GERMINATION

III.2.1 FACTORS AFFECTING GERMINATION OF UREDINIOSPORES

After dispersal of urediniospores onto a host, germination is the next stage of the life cycle. Knowledge of the factors affecting germination is important for glasshouse and laboratory pathogenicity work as well as for a general ecological understanding of the rust.

III.2.1.a Introduction

Scott (pers. comm. 1995a) experimented with a great number of factors that affected germination percentage (Section I.5.1.d). In the present study, the effects of the temperature and light regimes on the germination and germ tube growth of urediniospores were investigated on a water agar medium. The aim was to identify the conditions suitable for urediniospore germination for future inoculations and to assess the potential for urediniospore germination in New Zealand high country conditions.

III.2.1.b Materials and methods

III.2.1.b(i) Experiment I: Montpellier

The experiment was undertaken in incubator units at the CSIRO Unit of Biological Control, Montpellier, France, in November, 1993. The germination of urediniospores on water agar was monitored under six temperature regimes (5, 10, 15, 20, 25 and 30°C) and two light regimes (dark or light). Mature urediniospores of isolate BRIT32 were collected by a vacuum apparatus from *H. pilosella* plants (grown from seed of HpilHOs locality). The spores were released in a settling tower onto water agar (0.95%) resulting in a more or less even deposition of dry urediniospores (ca. 300 cm^{-2}). Sections of the water agar (1 cm^2) were immediately transferred to double thickness glass microscope slides in petri dishes lined with moistened filter paper. The microscope slides in petri dishes had been pre-incubated at the appropriate temperatures, reducing the lag between spore deposition and attainment of the desired experimental temperature for each treatment. Dimock and Baker (1951) recognised

the potential significance of such lags in attaining temperature in experiments designed to compare urediniospore germination at various temperatures.

The darkness replicates were sealed with plastic wrap and fully covered by aluminium foil. The light replicates were sealed with plastic wrap alone. Replicates were placed in the appropriate incubator each with constant light. Light levels were variable between incubators. The temperature at plate level was monitored in each incubator.

Percentage germination was assessed on five agar sections ten minutes after spore deposition on the agar. Fifteen replicates were made for each of the 12 temperature and light combinations. Three replicates were removed for each treatment at 2, 4, 8, 16, and 24h following inoculation. Lactophenol cotton-blue was placed on the surfaces of each replicate to halt germination and stain the germ tubes for observation. Percentage germination was calculated from 200 spores under randomly chosen fields under a light microscope. The length of ten randomly chosen germ tubes were recorded. If fewer than ten germ tubes were present in a replicate, all germ tubes were measured.

III.2.1.b(ii) Experiment II: Edinburgh

The germination of urediniospores was tested with another rust isolate, BRIT38, in June, 1992. Two ages of urediniospore were tested on either blocks or whole surfaces of water agar (0.95%) and either in glasshouse conditions or in a laboratory drawer. These conditions were similar to the two main inoculation conditions, those for detached leaves in the laboratory, and those for detached leaves and whole rosettes in the glasshouse. Urediniospores were collected from sori with a needle and were used either fresh or after 21 days storage at room temperature (ca. 17°C) in a brown paper envelope. Each agar block treatment was kept on a microscope slide separated from moist filter paper by glass rods in a petri dish. The agar surface treatments involved urediniospores being deposited on water agar in petri dishes. Urediniospore deposition onto agar was carried out with slightly moist cotton bud swabs of urediniospores. The petri dishes were covered. Half the prepared petri dishes was immediately placed in a drawer with darkness. The remaining half was kept on a

glasshouse bench (13°C daytime minimum, 10°C night-time minimum) ca. 5 hours before sunset without artificial lighting. After 16 hours, lactophenol cotton blue was dropped onto the surfaces and percentage germination was calculated on the basis of ca. 200 urediniospores.

For the purposes of calculating germination percentage, only urediniospores with a germtube extension of greater than one spore length was counted as germinated. For the calculation of average germ tube length in Experiment I, germ tube lengths included were at least 0.25 of a spore length.

III.2.1.c Results

III.2.1.c(i) Experiment I

No germination was seen on the agar sections observed ten minutes after spore deposition. Urediniospores germinated at all of the temperatures tested and in both light and dark. There were significant differences between treatments in percentage germination, rate of germination and germ tube growth. The results for percent germination over time are presented in Table 3.1 and for germ tube length in Table 3.2.

Table 3.1. The influence of temperature and light regime on percentage germination of urediniospores over time.

		Temperature (°C)						
Light Regime	**Time(h)**	**5**	**10**	**15**	**20**	**25**	**30**	**lsd[a]**
Light	2	0	1.7	0	0	2	0	1.1
	4	1.3	2.7	1.3	1.3	1	0	[b]2.5
	8	1.7	7.3	3.3	72	0.7	0	6.0
	16	17	33	4.3	70	0.7	0	24.7
	24	23	65	11	73	1.7	0	19.3
Dark	2	20	29	57	26	23	21	22.3
	4	12	31	40	41	46	19	22.4
	8	15	43	45	60	44	10	23.9
	16	22	44	44	72	51	13	14.7
	24	25	39	58	66	44	8.7	20.3

[a] The least significant differences are given for each time interval.
[b] The differences at time 4h in the light were not statistically significant (p=0.3843).

The significance of differences over the temperature range, in germination percentage (transformed, log percentage +1) and in germ tube length, in light and in dark was tested by analysis of variance (ANOVA) within each time interval. Significant differences shown in the tables were derived from Duncan's multiple range test and do not relate to differences between time intervals. Differences between means at all time intervals were highly significant (p=0.001) over the temperature range and also between light treatments.

Table 3.2. The influence of temperature and light regime on average germ tube length (μm) of germinated urediniospores over time.

		Temperature (°C)						
Light Regime	**Time(h)**	**5**	**10**	**15**	**20**	**25**	**30**	**lsd[a]**
Light	2	0	18	0	0	51	0	13
	4	64	59	38	20	29	0	41
	8	49	110	68	120	50	0	57
	16	190	190	140	210	25	0	120
	24	210	270	150	230	15	0	72
Dark	2	34	37	36	50	40	44	16
	4	120	78	110	73	92	90	36
	8	190	70	200	130	210	110	68
	16	350	120	340	80	210	96	140
	24	360	92	310	110	330	120	95

[a] The least significant differences are given for each time interval.

Germination was much more rapid in the dark, with optimum temperature for percentage germination being between 15 and 20°C. In dark, germ tube length was longest at 15 °C at the 4 and 8h time intervals. When comparing germ tube lengths at 10, 15 and 20°C, germ tubes were still significantly longer in the dark than in the light at 24 hr (p=0.0024). Germination percentage over all temperatures were still significantly higher in the dark after 24h (p=0.0001) but at that stage no statistical difference was evident between light regimes when comparing just 10, 15 and 20°C (p=0.0828). In the continuous light regime the differences between these three middle range temperatures were significant at 16h (p=0.0006).

In the 15°C, light treatment, germination was very low.

III.2.1.c(ii) Experiment II

An average of 96% of urediniospores germinated over all treatments. All treatments showed greater than 90% germination with no significant differences between treatments (Table 3.3).

Table 3.3. Influence of varying environmental conditions on germination of isolate BRIT38 urediniospores.

Environment	Storage	Agar Surface	% Germination
Laboratory	Fresh	Slide	92
	Fresh	Surface	96
	Stored	Slide	98
	Stored	Surface	98
Glasshouse	Fresh	Slide	99
	Fresh	Surface	94
	Stored	Slide	95
	Stored	Surface	95

III.2.1.d Discussion

III.2.1.d(i) Experiment I

Germination was much more rapid in continuous dark than in continuous light. In field conditions, a reasonable assessment of dew period would be closest to 8h or less, throughout most of the rust season. Therefore assessment of optimum conditions for urediniospore germination and germ tube growth could be taken at the 8h time. At that time, in continuous dark, the germination percentage was optimum at 20°C and the average germ tube length (including ungerminated urediniospores) was longest at 15°C. Germination percentage was relatively high and germ tube length was relatively long, over the whole temperature range of 10 to 20°C. Emge *et al.* (1981) selected 2 to 7h dew periods as the most relevant times to investigate for the *Chondrilla* rust system in California.

The three replicates of each treatment are valid for testing differences between light treatments, but they were pseudoreplicates in terms of testing between temperatures, because different incubators were used for each temperature. This was probably not important for comparing results of the continuous dark treatments as temperature was likely to be the only significant factor differing between treatments in different

incubators. The differences between temperature treatments in the continuous light samples may have been confounded by differing light levels between incubators, which may account for the aberrant germination rate of the 15°C, continuous light treatment in all three replicates. Eversmeyer and Kramer (1989) found that higher light levels significantly increased germination of urediniospores of *Puccinia recondita* and *P. graminis*.

III.2.1.d(ii) Experiment II

High germination rates were shown to be possible in the light and temperature regimes under which most inoculation experiments were conducted. The high percentages of germination compared very favourably with those achieved in Experiment I but too many factors (such as origin of isolates and methods of urediniospore collection and deposition) differed between the experiments to have determined if actual urediniospore viability was less in the Montpellier experiment.

Urediniospores of two species of rust, *Puccinia recondita* and *P. graminis*, exhibited greater and more rapid germination under a wider range of temperatures (Kramer and Eversmeyer, 1992) than the results presented here for *P. hieracii* var. *piloselloidarum*. Both *P. recondita* and *P. graminis* exhibited at least 95% germination in 2h at 6 to 28°C; there was inhibition of germination for at least 4h at 2°C, much reduced germination at 31°C and complete inhibition at 35°C. Kramer and Eversmeyer (loc. cit.) reported that the two species had different optimum temperatures for germ tube extension. The optimum temperatures for germination were similar to the 11-18°C optimum reported for *Puccinia chondrillina* by Emge *et al.* (1981).

The germination experiments were conducted in a humid environment whereas in the field a higher temperature would generally mean a higher rate of evaporation and potentially a shorter dew period. Furthermore, the temperature in which rust urediniospores are produced on the host can affect the requirements for subsequent germination (Park, 1990). The urediniospores used in Experiment I developed on hosts in a glasshouse with daytime temperatures of sometimes over 25°C, therefore the optimum temperature for germination of urediniospores produced in cooler field

conditions may be lower than the results presented here indicate. Scott (pers. comm. 1995a) reported optimum temperature for germination percentage for urediniospores of British *P. hieracii* var. *piloselloidarum* grown in cooler glasshouse conditions to be 14° to 18°C.

The optimum temperature range for germination and germ tube growth is not necessarily the optimum range for infectivity of a rust pathogen (Dimock and Baker, 1951). The results, however indicate that urediniospores germinate over a relatively wide range of temperatures and germination is faster in the dark.

III.3 OBSERVATIONS OF INFECTIVITY

III.3.1 INTRODUCTION

Throughout experimentation on rust pathogenicity, observations were made on the plant parts infected. Scott (pers. comm. 1995a) reported that older leaves were generally more susceptible to rust infection than younger leaves.

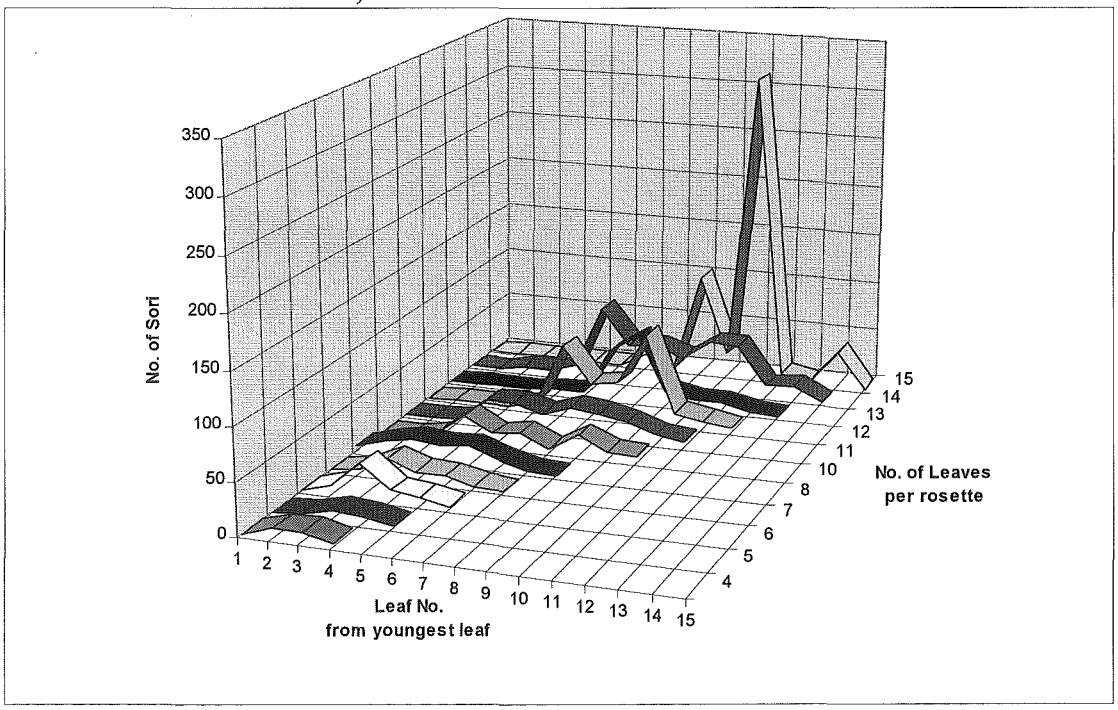
III.3.2 MATERIALS AND METHODS

Observations were made during the work on self spread of rust (Section III.4) and on the infectivity of seven rust isolates on New Zealand *H. pilosella* rosettes ex HpilHOs (Section V.1.3). General observations on the plant parts affected by rust were also made during other rust pathogenicity work.

III.3.3 RESULTS

Rust infection was observed on all leaf ages except for small unopened leaves. Infection was less common on younger leaves than on older leaves. The relative number of sori were recorded on leaves of different ages (1 = youngest open leaf at time of inoculation) on a range of immature (no flowering or stolons) *H. pilosella* plants with different numbers of leaves (Fig 3.19). These were the 121 plants that were infected in the series of inoculations in Section V.3.2. Infection was heaviest on rosettes with larger numbers of leaves. There were apparent differences between leaves of different ages in their susceptibility to trust with more infection generally occurring on older leaves, this could be seen most clearly on rosettes with larger numbers of leaves. In other inoculations and in work on the self spread of rust, leaves and rosettes produced on stolons were susceptible to rust but no infection was noted on floral parts.

Fig 3.19. Average number of sori. (On leaves of different ages on plants with different numbers of leaves).



III.3.4 DISCUSSION

Leaf age was shown to affect rust infection, with the youngest leaves exhibiting less sori. Although the small, young leaves may exhibit fewer sori due to decreased surface area, the magnitude of the differences with older leaves and the fact that often no sori were produced on very young leaves indicated that physiological or other factors may be involved. However, small leaves on stolons and leaves of rosettes produced at the end of stolons were reasonably susceptible to infection. The observations match those in the field where sori appear to be more common on older leaves. In the field, infection was often noted on stolon leaves, ramets and, occasionally, stolons themselves. No infection of floral parts was noted in the field but uredinia were observed on scapes of several glasshouse grown *H. pilosella* plants. No purposeful inoculations of floral parts were made. Rust (*P. hieracii* var. *hieracii*) sori have occasionally been observed on scapes of *Taraxacum* (pers. observation).

In glasshouse conditions rosettes exhibited high rates of leaf production (>1 new leaf per week) and senescence of old leaves (time from starting to yellow to fully brown: ca. 1-2 weeks). Whereas a high rate of leaf senescence in glasshouse conditions meant a high rate of loss of rust sori, the slower turnover rate of leaves in the field meant that a rust infected leaf could remain for most of the growing season.

III.4 SELF SPREAD OF RUST

III.4.1 INTRODUCTION

One of the main advantages of classical biological control of weeds is that the agent can spread throughout infested areas. The ability of *Hieracium* rust to spread onto *Hieracium* plants without inoculation was observed in glasshouse conditions. Two experiments were aimed at quantifying the spread of rust onto several genotypes of New Zealand *H. pilosella.*

The first experiment aimed to determine the distance to which infection might spread, from a row of isolate BRIT12 infected rosettes, over a uniform area of *H. pilosella* grown from seed of 12 New Zealand locality/genotype combinations. The second experiment tested the capacity of three rust isolates to spread to adjacent rosettes of five New Zealand locality/genotype combinations.

III.4.2 DISTANCE OF SPREAD

III.4.2.a Introduction

Glasshouse observations indicated that self spread of rust was occurring but the distance to which self spread could occur was unknown. An experiment was conducted to attempt to assess spread in terms of time and percentage of neighbouring rosettes infected. Selected localities of New Zealand *H. pilosella*, that had been subjected to taxonomic analysis, were used to check for differences in spread onto different genotypes.

III.4.2.b Materials and Methods

The experimental design was a randomised block design of 48 rows by 12 columns of 12 New Zealand *H. pilosella* host types, including nine localities, represented in each row and an additional row of rust infected plants placed at one end. New Zealand rosettes had been grown from seed (germinated on water agar, see Section II.3.1.a) of

the localities detailed on Table 3.4. All rosettes were ten weeks old and individually potted in 7cm by 7cm wide by 8cm deep pots. The area covered by the rosettes was thus 3.4m by 0.84m. The seed of nine of these localities was taken from the same capitula as seed used to raise stock for the isozyme electrophoresis work in St Andrews (Section VII.2.6.c). It was assumed that the vast majority, if not all, of the seed in these capitula was produced apomictically and therefore that the rosettes grown from the same capitulum were probably isogenic (genetically identical); the safety of this assumption was considered (Section VII.1.4). Information was available on the differences in isozyme patterns between some of the rosettes (Table 3.4). The hexaploid cytotype from locality HpilHAs was also included.

The rust infected row of plants were all rosettes with a heavy infection of isolate BRIT12 which were transplanted from the field eight weeks prior to the experiment. The experiment was watered daily from above, with water sometimes sitting on the rosette leaves overnight. Rosettes were monitored every second day for 13 weeks. At the first sign of rust sori, infected rosettes were transferred to another glasshouse and replaced with rosettes ex Holbrook. This was to ensure that new infections were always from the initial row of infected BRIT12 rosettes. No other rust isolates were present in the glasshouse used for this experiment. The experiment was conducted in Edinburgh from 15 December 1992 through to 9 March 1993. Glasshouse conditions were 13°C day time minimum, 10°C night-time minimum without artificial lighting.

III.4.2.c Results

The number of rosettes of each locality and genotype infected after 13 weeks of potential exposure to inoculum are given in Table 3.4. Analysis of variance and multiple regression found no significant difference in sori numbers or in infected plant numbers respectively, between the 12 localities even when corrected for the gradient of infection from inoculum source to the far end. There was no significant difference evident between columns either. The gradient of sori produced, from the inoculum source (1 row) to the far end (48 rows away) is shown in Fig. 3.20. The log transformed distribution approached a linear line with a gradient of -0.06

log(sori+0.001) for each row in terms of rows away from inoculum source (the change with distance from inoculum was highly significant, p=0<0.0001). The numbers of plants infected over time are shown in Fig. 3.21.

Fig 3.20. Self spread of rust isolate. (Isolate BRIT12 on New Zealand *H. pilosella* : number of sori produced on rows related to distance from infected rosettes).

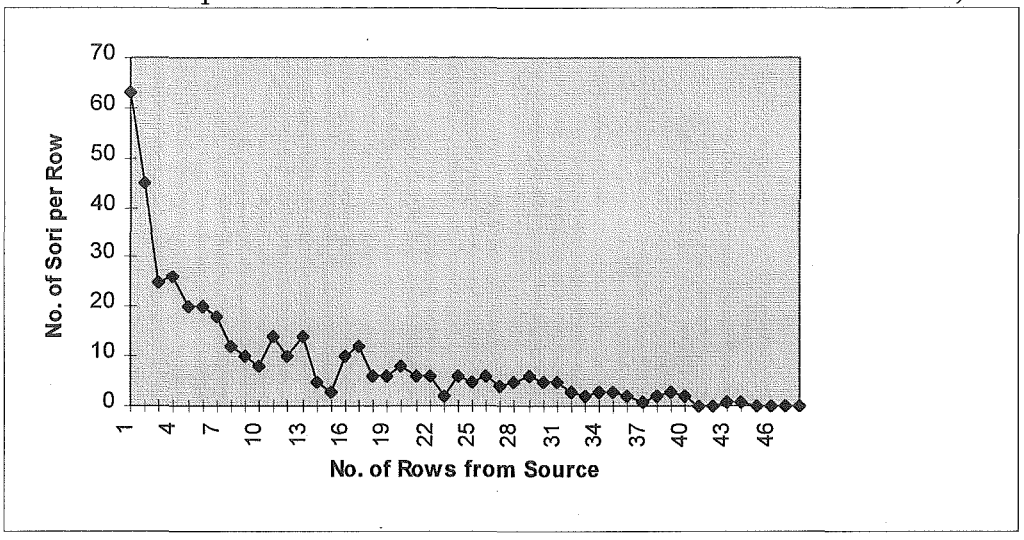
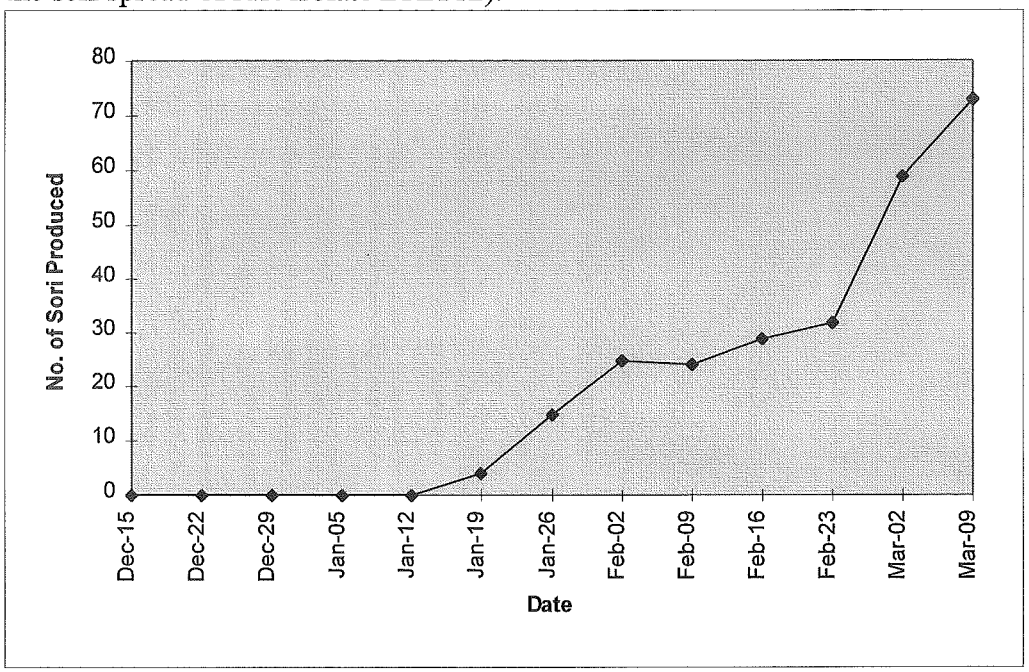


Table 3.4. Number of rosettes infected from each source locality over the duration of the self spread distance experiment.

Locality[a]	Percentage of Rosettes Infected
HpilHSp(i)	40
HpilCRa1 (t)	40
HpilLCo(i)	38
HpilLCl	46
HpilMJt(ii)	35
HpilHOs(ii)	29
HpilTWo	48
HpilMAs1(i)	50
HpilLRu(i)	33
HpilHAs5X(i)	38
HpilHAs6X(i)	48
HpilSCs1	42

[a] (t) = subsp. trichosoma (all rosettes from other localities were subsp. *micradenium*). Rosettes from localities with (i) or (ii) following the code were all grown from seed of the same capitulum and siblings of these rosettes were analysed with isozyme electrophoresis. Results of isozyme electrophoresis are given in Section VII.2.6.c.

Fig 3.21. Number of sori produced over time. (On New Zealand *H. pilosella* from the self spread of rust isolate BRIT12).



It was not until the week of 12th of January that infection was noted on the previously uninfected rosettes. There was a maximum of three times that any one position was infected by rust. The maximum spread of rust noted was to the 44th row of rosettes (3.1m). Most rosettes in the rows closest to the originally infected rosettes developed infection over the course of the experiment. Numbers of sori produced generally increased from that point with the highest numbers of sori produced per week being in the final week of observation (early spring), before termination of the experiment.

III.4.3 SELF SPREAD FROM THREE ISOLATES ON TO FIVE *H. PILOSELLA* REPRESENTATIVES

III.4.3.a Introduction

Differences were investigated between three rust isolates in their capacity for self spread over a range of New Zealand *H. pilosella*. The New Zealand hosts were from three localities; two were represented by two recognised genotypes. Differences were assessed between isolates in the percentage of rosettes infected and number of sori produced for each host type.

III.4.3.b Methods

Infected plants were placed in the centre of plastic cubicles and surrounded with rust free plants. The three isolates were represented by two infected plants in each of two 35 cm high translucent plastic cubicles. The isolates were BRIT12, BRIT32 and BRIT38. The isolates were all grown on New Zealand *H. pilosella* hosts of locality HpilHOs for this experiment. The rust free plants included two representatives of each of the selected New Zealand *H. pilosella* hosts detailed, including one from locality HpilCRa, two cytotypes from locality HpilHAs, and two genotypes from locality HpilHOs.

Ten week old rosettes were grown from seed from the five locality and genotype combinations detailed in Table 3.5. Seed used to grow the rosettes of the Haldon location were taken from the same capitula from both a confirmed pentaploid and an hexaploid. Seed from Holbrook was taken from the same capitula as the seed used to grow rosettes used in isozyme electrophoresis work (Section VII.2.6). Rosettes from the Craigieburn locality were grown from randomly collected seed. All seed had been germinated on water agar, using the method in Section II.3.1.a.

The selected New Zealand hosts were placed directly adjacent to the infected plants. Non-target species included in the host specificity experiments (Section III.8) were placed around the inside edges of the cubicle to check for non-specific self spread of rust.

III.4.3.c Results

All three rust isolates were observed to spread onto representatives of all five locality and genotype combinations. The number of sori and proportion of plants infected for each of the isolates on each of the host types are given in Table 3.5. Overall, 70% of rosettes became infected, and an average of 11.5 sori were produced on each rosette. There was a statistically significant difference between isolate BRIT38 and the other

two isolates (95% confidence level). No statistically significant difference was seen between localities in the number of sori produced. The interaction between isolate and host locality was not statistically significant so there was no evidence for differential susceptibility of the host locality/genotype combinations to the different rust isolates.

III.4.4 DISCUSSION OF SELF SPREAD OF RUST

The experiments demonstrated the ability of rust isolates to spread onto New Zealand rosettes of *H. pilosella* without artificial inoculations. The distance experiment demonstrated that this was possible over a distance greater than 3m in the absence of significant wind. There were no statistically significant differences between the susceptibility of the localities and genotypes of *H. pilosella* tested. In the second experiment isolate BRIT38 was noted as spreading to a lesser extent than the other two isolates.

Water splash could have been significant in spread of the rust over a short distance. Urediniospore dispersal would also have occurred via air currents. Although aphid levels were not high, individual aphids were observed to have urediniospores attached to their legs and bodies after walking over rust infected leaf tissue, and they may have aided in the dispersal of some urediniospores. Most infection occurred during the period of very late winter to early spring and was on the increase at the time that experiments were terminated due to the commencement of further field collections of rust isolates in the spring.

Table 3.5. Self spread of three British rust isolates on to New Zealand *H. pilosella* rosettes. (Average number of sori produced and percentage of rosettes infected.)

		Locality code of rosettes						
Isolate		HpilCRa	HpilHAs 5X	HpilHAs 6X	HpilHOs(i)[a]	HpilHOs(ii)[a]	Average	Significance[b]
BRIT12	no.of sori	14	13	9.5	15	29	**16.3**	a
	% rosettes	75	75	75	100	75	**80**	
BRIT32	no.of sori	20	9.3	9.8	13	18	**14.0**	a
	% rosettes	75	50	100	75	75	**75**	
BRIT38	no.of sori	4	3.5	3.3	6.3	4.5	**4.3**	b
	% rosettes	75	50	50	75	25	**55**	
Average	**no.of sori**	**12.7**	**26.1**	**22.6**	**34.1**	**17.4**	**11.5**	
	% rosettes	**75**	**58.3**	**75**	**83.3**	**58.3**	**70**	

[a] Rosettes from locality HpilHOs were grown from seeds of one of two capitula, (i) and (ii) - a rosette grown from seed of both of these capitula was included in isozyme electrophoresis and displayed probable genetic variation (Section VII.2.5).

[b] Statistical significance between the average number of sori produced by each isolate was derived from Duncan's multiple range test. Isolates with the same letter are not significantly different at the 95% confidence level.

The spread of the rust, *Puccinia recondita*, on wheat (*Triticum aestivum* L. cv. Ticonderoga) was demonstrated by Aylor (1987). When Aylor placed plants 3.7m downwind from infected plants for 2 to 4h and subsequently transferred the plants to a dew chamber, they always became infected with rust. Under the differing conditions of the present study, *Hieracium* rust was not observed to spread as efficiently as *P. recondita*.

In Edinburgh glasshouses, rust infected *Hieracium* plants were kept isolated from each other by space or walled cubicles of translucent plastic. Uninfected *H. pilosella* plants from New Zealand seed stock, and transplanted UK plants from locality "Bush" (see Table 2.8), together with plants of other genera being raised for host specificity testing, were placed between infected plants. Most plants were watered from above and rust was observed to infect some of the buffer plants of *H. pilosella* ex Bush and ex New Zealand. Secondary infection, i.e. increased infection on the same plant, was usual.

Self spread infection was not commonly observed in glasshouse conditions in Montpellier as watering was either from the base or carefully applied to the soil with limited wetting of the leaves. The relatively warm, dry conditions ensured that water did not remain for long on leaf surfaces in the Montpellier glasshouses.

The long distance dispersal capacity of urediniospores of other rust species have been demonstrated by such occurrences as the infection of New Zealand poplar trees with rust from Australia and the subsequent spread within a season to far eastern parts of the North Island (Kraayenoord *et al.*, 1974). *Puccinia chondrillae*, introduced for biological control of *Chondrilla juncea*, was reported to spread several kilometres a year in USA (Adams and Line, 1984) and up to 200 km after six weeks in Australia (Cullen *et al.*, 1973). In a study of the speed of spread of *Puccinia canaliculata* (Schwein.) Lagerh., a biological control agent of *Cyperus esculentus* L., Phatak *et al.* (1983) found that the rust spread from just one or two visible rust sori to infect all hosts within 1m by ten days; all hosts within 7m by 14 days and by 28 days, infection was found 130m away from the original source of inoculum. The capacity of long

distance spread by *P. hieracii* var. *piloselloidarum* is evidenced by the presence of rust on isolated patches of *H. pilosella* (pers. obs.).

Urediniospore deposition of *Uromyces appendiculatus* (Persoon) Unger (bean rust) was observed to have a rapidly decreasing gradient for the first one or two metres and then the differences were more gradual over longer distances (Aylor and Ferandino, 1990). Aylor and Ferandino (loc. cit.) emphasised that the source and crop geometry must be included in a model of spore dispersal. *H. pilosella* does not provide a high source for urediniospore liberation but the spread throughout a patch is facilitated by the low stature.

Environmental conditions were not controlled and although the higher levels of infection towards the end of the first experiment could have indicated a change in host condition this could not be proven.

III.5 REPRODUCTIVE FITNESS OF RUST ISOLATES

The reproductive fitness refers to the capacity of a pathogen to infect a range of genotypes (Shaner *et al.*, 1992). The range of *H. pilosella* genotypes and other *Hieracium* species that could be affected by certain rust isolates was investigated. This section includes the results of several isolates tested on a range of New Zealand *H. pilosella* populations over the two year period of study.

III.5.1 ISOLATE BRIT53 TESTED ON A VARIETY OF *H. PILOSELLA* ROSETTES.

As a preliminary investigation of the susceptibility of New Zealand *H. pilosella* from different localities to rust isolate BRIT53, inoculations were made onto three rosettes of each of five localities. The localities were HpilHOs, HpilLRu, HpilHAs, HpilCRa and HpilSCs. All rosettes were six weeks old at the time of inoculation. Inoculations were made with a moist cotton bud according to the methods of Section II.2.4.b. Rosettes were monitored for four weeks for any production of sori (Table 3.6).

Table 3.6. Results of inoculation with rust isolate BRIT53 on five localities of New Zealand *H. pilosella* (no. of sori).

	Rosette		
Locality	**1**	**2**	**3**
HpilHOs	107	54	0
HpilLRu	25	0	0
HpilHAs	16	0	0
HpilCRa(i)	0	0	0
HpilSCs(i)	0	0	0

One rosette was infected from localities HpilLRu and HpilHAs. Two rosettes from locality HpilHOs were infected and these both exhibited the highest numbers of sori. No infection was seen on any of the rosettes from HpilCRa or HpilSCs. Thus only 27% of rosettes were infected by the isolate. On the basis of higher levels of susceptibility, locality HpilHOs was selected as the source of hosts for the comparison of several rust isolates in Section V.1.3.

III.5.2 RESULTS OF RUST ISOLATE INOCULATIONS ON TO A RANGE OF *H. PILOSELLA* POPULATIONS

Over the course of study, a large number of rust isolates from throughout Europe were inoculated on a range of New Zealand *H. pilosella* populations. Many of these inoculations were part of the screening process and others were made when inoculum was available. The results of inoculations with several of the isolates are presented in Table 3.7. In some cases, two sibling rosettes were tested, and only one became infected. This result was treated as one out of two in the table despite the likelihood of the siblings being isogenic (genetically identical).

Infectivity was generally much lower in Montpellier experiments than in Edinburgh experiments. Indications were that the problem lay in host condition rather than inoculation conditions as infections were occasionally severe. Host condition affecting infection could be genetic or environmentally modified, or both. To ascertain the importance of host genetics, host taxonomic work was undertaken

(Chapter 7) and a range of genotypes and populations of New Zealand *H. pilosella* were tested for susceptibility to isolate ÉIRE14 (Sections III.5.3 and III.5.4).

Rosettes grown from seed from one locality (HpilGPs) displayed very little susceptibility to most rust isolates (Table 3.7 and Section V.3.3). The susceptibility of seedlings from this population was investigated in more detail (Section III.6).

The ranking of isolates in terms of infectivity was not taken from Table 3.7 but rather from the screening process in Chapter 5 where comparisons between isolates were made at the same time on similar host plants. Nevertheless, the values in Table 3.7 indicate that several of the Irish isolates had a relatively high infectivity.

Table 3.7. Infection of *H. pilosella* host localities by several rust isolates. (No. times infected / no. times tested)

	Locality																		
	Hpil HSp	Hpil CRa	Hpil LLy	Hpil LCo	Hpil LRd	Hpil GPe	Hpil MJt	Hpil HOs	Hpil MAs	Hpil LRu	Hpil TWz	Hpil HAs	Hpil HAs	Hpil SCs	Hpil THs	Hpil LCl	Hpil LTe	total	% rosettes infected
Isolate		1							1			5X	6X	1					
Ireland																			
ÉIRE14	2_2	2_2	2_2	1_2	3_4	2_14	4_6	10_13	1_2	5_6	56_118	5_6	2_2		1_17			71_146	49
ÉIRE29					2_2	0_2	1_2	2_2		2_2	1_1	5_7						13_18	72
ÉIRE32	5_5	4_5			2_2	0_2	1_2	2_2		2_2		3_5	5_5				3_5	27_35	77
ÉIRE45			0_1		2_2	0_2	2_2	2_2		1_2		5_5	4_5					16_21	76
ÉIRE66	5_5	3_5			2_2	0_2	5_7	6_7		5_7	5_5	4_5	5_5			4_5		44_55	80
Britain																			
BRIT2								12_25										12_25	48
BRIT12					1_2	0_6	1_2	90_131		2_2	4_4							98_147	67
BRIT32		1_6	2_10		2_2	2_32	1_2	20_27		1_2	5_45			2_7			0_36	36_169	21
BRIT38						2_24		19_25										21_49	43
BRIT52								21_25										21_25	84
BRIT53		0_3						21_27		0_3		1_3		1_3				23_39	59
BRIT55						0_2		15_25										15_27	56
SELF1								16_25										16_25	64
Europe																			
FRAN4								2_4			2_2							4_6	67
FRAN262								1_4			2_2							3_6	50
SVER341								3_4			2_2							5_6	83
Total																			

III.5.3 PATHOGENICITY OF ISOLATE ÉIRE14 ON DETACHED LEAVES OF A RANGE OF *H. PILOSELLA*

III.5.3.a Introduction

The ability of isolate ÉIRE14 to infect *H. pilosella* from 21 New Zealand localities of single populations from Chile, Scotland and Sweden was tested using detached leaves. The test was conducted as a preliminary assessment of whether certain New Zealand localities of *H. pilosella* would require special attention as to their susceptibility to ÉIRE14.

The New Zealand *H. pilosella* locality collections were usually from a relatively large area, around 0.25 hectares, thus there was a potential for genetic variation. Isozyme electrophoresis(Section VI.2) demonstrated genetic variation within some of these *H. pilosella* populations (Section VII.2.6).

III.5.3.b Methods

The experimental design was a single rust isolate (ÉIRE14) tested on a detached leaf of each of three rosettes for each of 24 *H. pilosella* host localities. A leaf was excised from each of three randomly chosen rosettes of each locality to be tested. Inoculation method involved moist cotton buds as described in Section II.2.4.a. Two extra leaves from each rosette were included as controls and brushed with a moist cotton bud lacking rust spores. The localities are listed in Table 3.8.

The Chilean rosettes were sent by mail from Southern Chile, representing one population. The Swedish rosettes were transplanted from one site in Southern Sweden, collected as host of rust isolate SVER322. The Bush, Scotland rosettes were transplanted from a single patch. Rosettes representing the New Zealand localities were grown from seed collected in the field. All rosettes had been grown in the glasshouse for at least ten weeks.

III.5.3.c Results

Infection was produced on detached leaves of every locality. The Chilean and Swedish detached leaves were all infected. Two detached leaves of the Scottish population were infected. Apart from the HpilGPs locality, two or three leaves of all remaining New Zealand populations were infected by rust. No control leaves exhibited rust infection.

Table 3.8. Infection of detached leaves of *H. pilosella* from various populations inoculated with rust isolate ÉIRE14.

Source of *H. pilosella*	No. leaves infected (max. 3)	Average no. of sori
CHILE	3	33
BUSH	2	19
SÄVSJÖ	3	23
HpilCRa	3	10
HpilDKc	2	10
HpilGPe	1	4
HpilHSp	3	22
HpilHEr	3	24
HpilJPa	3	20
HpilLCl	3	16
HpilLCo	3	21
HpilLLy	2	23
HpilLRu	2	9
HpilLTe	3	26
HpilLRd	3	17
HpilMAs	2	12
HpilMJs	3	14
HpilMJt	3	50
HpilODs	3	10
HpilSCs	2	8
HpilTHs	2	23
HpilTWo	3	13
HpilTWz	3	37

III.5.3.d Discussion

While the results did not rule out the possibility that resistant plants of *H. pilosella* exist within New Zealand populations, it was demonstrated that the majority of plants were susceptible to rust isolate ÉIRE14 under detached leaf inoculation conditions. *H. pilosella* from the HpilGPs locality was a particular concern and received further attention, although the infection of one detached leaf showed that at least some of the plants from that locality were susceptible.

Isolate ÉIRE14 was also tested on a range of *H. pilosella* whole rosettes (Section III.5.4) and also specifically on HpilGPs rosettes and detached leaves (Section III.6).

III.5.4. PATHOGENICITY OF ISOLATE ÉIRE14 ON WHOLE ROSETTES OF A RANGE OF *H. PILOSELLA*

III.5.4.a Introduction

A range of *H. pilosella* from New Zealand and other countries was tested for susceptibility to rust isolate ÉIRE14. The range included plants grown from seed of the same capitula as the seed of 17 sample plants analysed in isozyme electrophoresis (Section VII.2). The aim was to assess the host range of the rust isolate within *H. pilosella.*

III.5.4.b Materials and Methods

Two rosettes of each of 20 potential host types were inoculated with isolate ÉIRE14. The testing was conducted in two parts. Thirteen host types (marked 'a' in Table 3.9) were tested in Edinburgh glasshouses. These hosts included the siblings of plants included in the isozyme electrophoresis work at St Andrews University (See Section VII.2.6.c). Two rosettes grown from seed of a capitulum with the characteristics of *H. pilosella* subsp. *trichosoma* were also inoculated. In addition, two rosettes, transplanted from the field, from each of localities BUSH (Scotland), CHILE (South

America) and SÄVSJÖ (Sweden) were included. Inoculum was taken from *H. pilosella* plants ex HpilHOs, infected with isolate ÉIRE14.

The seven remaining hosts were inoculated at Montpellier. These were all siblings of plants that were included in isozyme electrophoresis studies. Inoculum was taken from an original *H. pilosella* host plant of isolate ÉIRE14.

Inoculation was made with cotton buds according to the methods in Section II.2.4.b. Rosettes were monitored for the production of sori after four weeks. At that time, if sori were not produced on either of two rosettes of a host type, the inoculation was repeated.

III.5.4.c Results

Sori were produced on all host types, though not on all of the rosettes. No signs of resistance were observed. Two of the host types, HpilHAs(ii) and HpilLRd were apparently not infected by the first inoculation but one rosette of each type was infected after reinoculation.

III.5.4.d Discussion

The results demonstrated that isolate ÉIRE14 was able to infect representatives of the entire range of *H. pilosella* tested. The reason why some siblings were infected and not others was not easily explained. Genetic variation between siblings was unlikely (see Section VII.1.4) and the rosettes were grown in similar glasshouse conditions.

Table 3.9 Infection of locality / genotype combinations of *H. pilosella* by isolate ÉIRE 14. (Average number of sori and number of rosettes infected)

Host Code	Average no. of sori	No. of rosettes infected (out of 2)
HpilCRa1[b]	26	2
HpilHAs(i)[a]	91	2
HpilHOs(i)[a]	23	1
HpilHOs(ii)[a]	62	2
HpilLCo(i)[a]	3	1
HpilLRu(i)[a]	73	2
HpilLRu(ii)[a]	27	1
HpilMAs1[a]	107	2
HpilMJt(i)[a]	12	1
HpilMJt(ii)[a]	148	2
HpilHAs6X[a]	146	2
HpilHAs(ii)[b]	27	1[c]
HpilHAs(iii)[b]	78	2
HpilHSp[b]	7	1
HpilLLy[b]	30	2
HpilLRd[b]	8	1[c]
HpilTHs[b]	9	1
CHILE[a]	62	2
BUSH[a]	107	2
SÄVSJÖ[a]	8	2

[a] Inoculated in Edinburgh.
[b] Inoculated in Montpellier.
[c] one rosette of each location was only infected after reinoculation of both rosettes.

III.6 PATHOGENICITY ON *H. PILOSELLA* EX GODLEY PEAKS (HpilGPs)

III.6.1.a Introduction

Locality HpilGPs detached leaves had demonstrated limited susceptibility to British rust isolates in earlier phases of the project, with only two isolates causing infection during large-scale screening (see Section V.3.3). The extent to which the lack of infectivity was due to genotypic characters required testing. This included a comparison of infectivity with that on detached leaves from another locality, with detailed observations made of the infection process. Further experiments, with several rust isolates, were conducted on different batches of *H. pilosella* seedlings (ex HpilGPs) to assess the level of susceptibility.

III.6.1.b Methods

I.6.1.b(i) Detached leaves

Rosettes of Godley Peaks and Twizel collections were grown from seed. For both localities, 16 glasshouse grown rosettes were used, including eight one year old rosettes and eight four month old rosettes each. The rosettes from each locality had been grown in similar glasshouse conditions near to each other but not interspersed. A series of inoculations were made over 15 days beginning 15 September, 1993. On each day, four leaves of each locality were excised from different rosettes, two leaves from each age of plant. Leaves were chosen from the fourth to the seventh open leaves from each rosette. Inoculation was made with cotton bud as described in Section II.2.4.a and petri dishes were kept in glasshouse conditions (natural light/darkness, ca. 13°C daytime minimum, 10°C night time minimum). The leaves were inoculated urediniospores of isolate ÉIRE14. The production of sori was recorded. After 15 days, small sections of leaf tissue were examined under SEM and the remainder of the leaves was examined using the leaf clearing technique of Bruzzese and Hasan (1983).

III.6.1.b(ii) Infection of one year old whole plants

Twenty rosettes (one year old) grown from seed ex Godley Peaks were inoculated with a rust isolate BRIT12 on 15 September, 1993. Inoculation was carried out by brushing the leaves with cotton buds, previously brushed over uredinia on several leaves of the original host of BRIT12. A further 20 rosettes were treated as controls using moist cotton buds free of urediniospores. Inoculation was made with cotton buds as described in Section II.2.4.b and plants were kept randomly intermingled in glasshouse conditions (natural light/darkness, ca. 13°C daytime minimum, 10°C night time minimum) afterwards. The rosettes were monitored for production of sori.

III.6.1.b(iii) Infection of four month old whole plants

Inoculations were made on whole rosettes grown from seed from Godley Peaks and Twizel. The rosettes had been sown at the same time on randomly placed, replicated water agar plates and transferred at early cotyledon stage to individual pots. The pots

were filled with 1 part sieved soil (Montpellier CSIRO standard): 1 part sphagnum peat. The rosettes were grown randomly intermingled. Three months after seed sowing, forty rosettes were randomly selected from each location.

Urediniospores of isolate ÉIRE14 were inoculated onto eight rosettes of each locality. A further 24 rosettes of each locality were inoculated with urediniospores ex isolate Alcove (I). Plants were inoculated by brushing with cotton buds previously brushed over uredinia on several leaves of pentaploid rosettes ex HpilHAs, and the controls (8 rosettes per locality) were rubbed using moist cotton buds free of spores. Inoculation was made with cotton buds as described in Section II.2.4.b. Afterwards, rosettes were placed, randomly intermingled, in glasshouse conditions (natural light/darkness, ca. 13°C daytime minimum, 10°C night time minimum).

III.6.1.c Results

III.6.1.c(i) Detached leaves

Inoculations were successful on all Twizel detached leaves but no sori were observed on any of the Godley Peaks detached leaves. The observations of sori production on Twizel detached leaves are presented in Table 3.10. The shortest latent period observed was eight days, most were ten days and the longest was 12 days. There was no statistically significant difference between the number of sori arising on the different ages of Twizel rosettes from which the leaves were detached.

Table 3.10. Results of urediniospore inoculations on Godley Peaks and Twizel detached leaves. (number of sori produced on rep. 1, rep. 2)

Day of Inoculation[a]	Twizel				Godley Peaks			
	4 months old		12 months old		4 months old		12 months old	
	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2	rep 1	rep 2
1	22	35	15	22	0	0	0	0
2	26	33	36	25	0	0	0	0
3	15	27	20	29	0	0	0	0
4	27	28	12	18	0	0	0	0
5	14	29	15	32	0	0	0	0
6	2	4	1	2	0	0	0	0

[a] 1 = first day of inoculation, 2 = second day etc.

No visible resistance reactions, e.g., hypersensitivity, were seen on the Godley Peaks detached leaves. SEM and light microscope studies confirmed the formation of appresoria over stomata of the Godley Peaks detached leaves and some penetration. However there was no evidence of intercellular growth of the rust or necrosis of the host cells near the stomata. Leaf clearing of Twizel leaves showed that all leaves inoculated three or more days before microscopic examination had evidence of infection and intercellular mycelial growth.

III.6.1.c(ii) Infection of one year old whole plants

There was no evidence of infection on inoculated plants until on the 20th of October (35 days post-inoculation); one plant developed 11 sori. The next day a further two sori erupted on the same plant. No sori were observed on any of the other plants, including controls.

III.6.1.c(iii) Infection of four month old whole plants

Infection resulting from the inoculations was not severe in terms of the percentage of plants infected or the number of sori produced. The production of sori on each treatment is outlined in Table 3.11. Generally more rosettes of Twizel locality were infected with more sori than of Godley Peaks locality. Sori were observed on 25% of the isolate ÉIRE14 inoculated Godley Peaks rosettes and on 8% of Godley Peaks rosettes inoculated with isolate Alcove(i) compared to 50% and 21%, respectively, for Twizel rosettes. No resistance reactions were visible on any of the inoculated plants.

Table 3.11. Production of sori on Godley Peaks and Twizel localities' rosettes, inoculated with rust isolates ÉIRE14 and Alcove (I).

Isolate	Host Locality	No. of Plants	% Plants Infected	Average no. of Sori (per infected plant)	Latent Period[a]
ÉIRE14	Twizel	8	50	10.5	12
ÉIRE14	Godley Peaks	8	25	7.5	13
Alcove (I)	Twizel	24	21	5.6	22
Alcove (I)	Godley Peaks	24	8	3.5	22
Control	Twizel	8	0	0	-
Control	Godley Peaks	8	0	0	-

[a] Latent period (in days) of the first sori observed for each isolate, locality combination.

III.6.1.d Discussion

III.6.1.d(i) Detached leaves

Examination of the infection process could not elucidate the nature of the apparent resistance of Godley Peaks material although stomatal penetration by the rust had occurred on HpilGPs leaves. Resistance was present in several Godley Peaks plants of each of two separate batches, one eight months older than the other. The inoculation of Twizel detached leaves was successful with every leaf displaying intercellular mycelial growth three days after inoculation, and if left for long enough for production of uredinia, there was an average of 25 sori per leaf.

III.6.1.d(ii) Infection of one year old whole plants

If the observed infection on a Godley Peaks plant resulted from the experimental inoculation then the latent period of 35 days seems extraordinarily long. A latent period of 30 days has been observed for *Puccinia hieracii* var. *hieracii* on *Taraxacum officinale*, though only on a few occasions and always at a low (ca. 10°C) incubation temperature (pers. obs.). The twenty plants had been kept in normal glasshouse conditions in which the longest latent period during September/October was 21 days and most latent periods were observed to be less than 16 days. It is still possible that contamination may have occurred from wind carried spores or from insect or human vector. The plants had been two metres from the nearest rust isolates. They were inside cubicles with 35 cm high walls of translucent polypropylene plastic, but the cubicles were uncovered.

III.6.1.d(iii) Infection of four month old whole plants

The most important outcome of this experiment was the infection of four Godley Peak rosettes from a new batch of seedlings, including 25% of those inoculated with isolate ÉIRE14. Infection from most inoculations made at this time in Montpellier was generally low. Despite this, differences were apparent in the greater infection of Twizel compared to Godley Peaks plants. The superior infectivity of isolate ÉIRE14 compared to isolate Alcove(I) was also demonstrated again. The differences were not tested for significance because the levels of infection were so low.

A proportion of the Godley Peaks plants were demonstrated to be susceptible to isolate ÉIRE14, BRIT32 and BRIT12.

III.7 PATHOGENICITY ON *HIERACIUM* SPECIES - OTHER THAN *H. PILOSELLA*

III.7.1.a Introduction

The rust *P. hieracii* var. *piloselloidarum* is described as specific to the subgenus *Pilosella*, and has been recorded on a variety of taxa from this subgenus. Probst (1909) described races within this rust, all of which apparently had a limited host range within the *Pilosella* taxa that he experimented with (see Section I.4.2.b). In more recent experiments, Scott (pers. comm. 1995a) found that the rust from *H. pilosella* could infect other species but that *H. caespitosum* was not infected by any of the rust isolates tested (see Section I.5.1.b).

This series of experiments aimed to investigate the ability of rust isolates from several subgenus *Pilosella* spp. to infect the detached leaves of the range of taxa of *Hieracium* present in New Zealand.

III.7.1.b Methods

Detached leaves were collected from plants grown from seed collected in New Zealand. The locality descriptions and ploidy levels for the representatives of each taxa are given in Tables 2.6, 2.7 (localities) and Appendix 4, Tables A.5 and A.6 (ploidy levels).

H. aurantiacum : Only one locality (HaurPPa) of *H. aurantiacum* was used in the rust experiments. The ploidy level was tetraploid.

H. caespitosum : Five New Zealand collections (HcaeLCl, HcaeMJt, HcaeDKc, HcaeLRd, HcaeMAs) were included in the rust experiments. All of these collections matched subsp. *caespitosum*, the only subspecies recorded in New Zealand.

H. lepidulum : *H. lepidulum* was the only species, collected from New Zealand, of the subgenus *Hieracium* that was included in rust experiments. Seedlings of collection 'HlepLRd' were used. Plants were found to be triploid.

H. pilosella : Just one locality (HpilTWz) was used in these experiments.

H. praealtum : Two collections of this species were tested for susceptibility to the rust. One of the collections was a tetraploid subsp. *thaumasium* (HpraCRa), the other, a pentaploid subsp. *praealtum* (HpraLCl).

H. x *stoloniflorum* : This taxon is a hybrid of *H. aurantiacum* X *H. pilosella.* Three collections of *H.* x *stoloniflorum* (HstoCRa, HstoLTe, HstoMJh) were included in the detached leaf experiment.

Detached leaves of each New Zealand *Hieracium* taxon were placed on water agar (0.95%) in pairs; one of each pair was inoculated with a rust isolate using a moist cotton bud according to the methods in Section II.2.4.a, whilst the other leaf was treated with a moist cotton bud lacking urediniospores, as a control.

Seven rust isolates were collected from *H. flagellarum*, two from *H. praealtum*, and one from *H.* x *stoloniflorum*. Each of these rust isolates was tested on 10, 20 or 50 detached leaves of each of the representatives of the New Zealand *Pilosella* taxa mentioned above. Various numbers of isolates collected from *H. pilosella* were tested against the *Pilosella* species and also *H. lepidulum.*

Once isolate ÉIRE14 (from Ireland) was selected as the most promising rust isolate for New Zealand *H. pilosella*, the isolate was inoculated onto two whole rosettes of each of the taxa described above. The small scale experiment was designed to identify *Pilosella* species not infected by the rust isolates. Inoculation was made using moist cotton buds according to the methods of Section II.2.4.a with urediniospores from the original, transplanted, Irish *H. pilosella* hosts. Inoculated plants were monitored for four weeks post-inoculation for the production of sori or signs of resistance.

III.7.1.c Results

III.7.1.c(i) Cross infection between host species: detached leaf work

There was no evidence of infection on *H. lepidulum* or *H. caespitosum*. Three species, *H. pilosella*, *H. praealtum*, and *H.* x *stoloniflorum* were all infected by each of the rust isolates tested. *H. aurantiacum* was infected by all isolates tested but infection was weak and infrequent. No isolate infected more than 80% of the leaves of a species tested (Table 3.12). The sori on *H. aurantiacum* were very small (<0.25 mm diameter), few in number on a leaf, and were surrounded by chlorotic halos.

The inoculated host species had a highly significant effect on the result of inoculation ($p<0.001$, by analysis of variance). The original host species of the inoculum had no statistically significant effect ($p=0.057$) and differences between isolates taken from the same host species were insignificant ($p=0.318$).

III.7.1.c(ii) ÉIRE14 inoculations on whole rosettes

ÉIRE14 urediniospores produced sori on both plants of three species, *H. pilosella*, *H. praealtum* and *H.* x *stoloniflorum*. No sori were produced on the other three species, although a hypersensitive reaction (flecking) was seen at two infection points on one *H. caespitosum* plant. Table 3.13 presents the results including the number of sori produced on each of the infected plants.

Table 3.12. Cross infection between rust isolates from European *Hieracium* taxa on to detached leaves of New Zealand taxa. (percentage of leaves hosting sori)

	Inoculated onto ...						
Original Host (no. of isolates)	*H. aurantiacum* HaurPPa	*H. caespitosum*[a]	*H. lepidulum* HlepLRd	*H. pilosella* HpilTWz	*H. praealtum* HpraCRa	*H. praealtum* HpraLCl	*H.* x *stoloniflorum* HstoCRa
H. flagellarum (7)	5.3	0[c]	0	66[b]	17	13	51[b]
H. praealtum (2)	3	0[c]	0	60[b]	15[b]	10[b]	55[b]
H. x *stoloniflorum* (1)	8[c]	0[c]	0	70[b]	30	35	60[b]

[a] For each isolate, the 20 test rosettes consisted of four rosettes of each of the five *H. caespitosum* types investigated.

[b] and [c] indicate that there were ten or 50 replicate leaves, respectively, tested for each of the marked cross infections. Other cross infection tests involved 20 replicate leaves.

Table 3.13. Infection of whole rosettes of *Hieracium* spp. by isolate ÉIRE14.

Species	Code	No. of Sori (rosette 1, rosette 2)
H. pilosella	HpilTWz	14,21
H. aurantiacum	HaurPPa	0,0
H. caespitosum	HcaeMJt	0[a],0[a]
H. lepidulum	HlepLRd	0,0
H. praealtum ssp. *thaumasium*	HpraMJt	6,2
H. x *stoloniflorum*	HstoCRa	15,5

[a] Hypersensitive reaction (chlrotic flecks) were observed on the leaves of these plants and there was no spore formation.

III.7.1.d Discussion

These results confirm the cross infectivity of rust between *H. pilosella*, *H.* x *stoloniflorum*, and *H. praealtum* as reported by Scott (pers. comm. 1995a).

III.7.1.d(i) *H. aurantiacum*

Although very occasional infections did occur on some 1% of detached leaves, the symptoms indicated resistance of the host to the rust isolates. No rust infection was observed on *H. aurantiacum* in the field from a total of 87 localities during European collection tours. Rust has been recorded on *H. aurantiacum* in Europe (Gaümann, 1959) and on naturalised plants in the USA (Farr *et al.*, 1989). The reason for the weak reaction on *H. aurantiacum* requires further research, but the species is not considered to be a very serious weed problem in New Zealand (Hunter, 1991).

III.7.1.d(ii) *H. caespitosum*

This species never hosted sori throughout the duration of this study. Suitable isolates for *H. caespitosum* infection would be of interest as this species is on the increase in New Zealand and already poses weed problems in Marlborough and Otago (Hunter, 1991).

III.7.1.d(iii) *H. praealtum*

Three collections of this species were tested for susceptibility to the rust. Two of the collections were subsp. *thaumasium*, one was tetraploid from Craigieburn and the other was pentaploid from the Mt John trial site. A pentaploid subsp. *praealtum* collection from Lake Clearwater was the third. Plants from three locations were found to be susceptible to the rust.

III.7.1.d(iv) *H.* x *stoloniflorum*

This taxon is a hybrid of *H. aurantiacum* X *H. pilosella*. Susceptibility to rust infection was demonstrated. The sori were often large and percentages of leaves and rosettes infected were similar to those of *H. pilosella*. The taxon was evidently more

susceptible to the rust collections than was the *H. aurantiacum* collection. Three collections of New Zealand *H.* x *stoloniflorum* were included in the experiments, all showing similar susceptibility to the rust. All three collections were hexaploid (Section VII.1.3.c).

III.7.1.d(v) *H. lepidulum*

This species was the only species from New Zealand of the subgenus *Hieracium* that was included in rust experiments. The subgenus is not susceptible to the rust pathogen *P. hieracii* var. *piloselloidarum*, and results here on *H. lepidulum* confirmed this. There were no signs of infection or even visible resistance symptoms. Appresoria were sometimes formed above the stomata but no further development was noted.

Limited experimentation was conducted with *P. hieracii* var. *hieracii* isolates from *H. murorum*, *H. sabaudum* and *H. sylvaticum* hosts collected from Britain. They were inoculated onto *H. lepidulum* (HlepLRd) No sori were produced after inoculation with urediniospores according to the methods of Section II.2.4.a.

III.8 HOST SPECIFICITY TESTING OF RUST

III.8.1.a Introduction

Host specificity testing of the rust pathogen was undertaken on a number of important New Zealand plant species. These were primarily from the tribe Lactuceae, to which *Hieracium* belongs.

There are seventeen genera within the Lactuceae tribe that have been recorded in New Zealand. Some genera are represented by native species and these include *Embergeria* (Kirk) Boulos, *Kirkianella* Allan, *Microseris* D. Don, *Sonchus* L., *Taraxacum* G. Weber and possibly *Picris* L. (the possible native representative of the latter genus, *Picris hieracioides*, was described by Allan (1961) as a doubtful native). The two important

economic species in the Lactuceae tribe are *Lactuca sativa* L. (lettuce) and *Cichorium intybus* L. (chicory). *Tragopogon porrifolius* L. (salsify) is a less common crop. Many adventives, some causing weed problems, belong to this tribe including species of *Chondrilla* L., *Crepis* L., *Hieracium*, *Hypochoeris*, *Lactuca* L., *Lapsana* L., *Leontodon* L., *Mycelis* Cass., *Picris*, *Sonchus*, *Taraxacum*, *Tolpis* Adans., and *Tragopogon* L.

III.8.1.b Materials and methods

Rust isolates were inoculated onto a range of non-target species over a period of one and a half years in Edinburgh glasshouses. Leaf surfaces were brushed with moist cotton buds which had previously been brushed over uredinia of several leaves of original host rosettes. Initial testing was on ten New Zealand native and adventive species of the tribe Lactuceae, and control rosettes of New Zealand *H. pilosella* (ex HpilHOs), with British isolates BRIT12 and BRIT32. The native species tested were *Embergeria grandifolia* (Kirk) Boulos, *Kirkianella novae-zelandeae* (Hook. f.) Allan, *Microseris scapigera* (Sol. ex. A. Cunn.) Sch. Bip. and *Sonchus kirkii* Hamlin. The adventive species tested were *Crepis capillaris*, *Hieracium lepidulum*, *H. murorum*, *Hypochoeris glabra*, *H. radicata* and *Taraxacum officinale*. Isolate BRIT12 was inoculated onto five whole plants and ten detached leaves of these species on 20 occasions. Isolate BRIT32 was inoculated onto five whole plants and ten detached leaves on five occasions. In detached leaf experiments a variety of leaf ages was tested. Test plant ages ranged from one to ten months for each species. Rust inoculum was collected from infected original host plants in glasshouse conditions. The experiments were monitored for two months for any production of sori.

Subsequent host specificity testing was conducted in Montpellier glasshouses and included the above test species apart from *H. murorum*. In addition, three economic species of the Lactuceae, *Cichorium intybus*, *Lactuca sativa* (cos and red sails cultivars) and *Tragopogon porrifolius* were included. The group also included eight economic species of other tribes or families (detailed in Table 3.14). The inoculum was from rust isolates, ÉIRE14 and ÉIRE4, grown on New Zealand *H. pilosella* ex HpilHOs. Control rosettes were *H. pilosella* ex HpilTWz.

Detached leaves were tested for susceptibility to the isolate ÉIRE14 when the plants were seven weeks old. Ten detached leaves were selected from each species (and from both cultivars of lettuce) and from the control plants. Species with large leaves were represented by leaf sections of at least 2 cm^2. Inoculations were carried using moist cotton buds, as described in Section II.2.4.a, except that the experiment was carried out in a climatic chamber (ca. 20°C, 8h light, 16h dark); petri dishes were kept in covered humidity chambers for 24h post inoculation; and subsequently petri dishes remained in uncovered humidity chambers. The above modification of the method of inoculation was to avoid drying out of the agar which commonly occurred when petri dishes were kept in Montpellier glasshouses or incubators.

The whole plant experiments involved five plants of each species (and of both cultivars of lettuce) and five plants of control rosettes. Inoculum for the first experiment was ÉIRE4 and for the second experiment was ÉIRE14. All upper and lower leaf surfaces of most plants were inoculated in each experiment. Large leaved plants such as sunflower and many leaved plants such as the grasses were, however, only made on a portion of some leaves. Incubation conditions were as described in Section II.2.4.b.

In Montpellier, inoculated hosts were monitored for two months for any production of sori. Leaf segments of 1 cm^2 were taken from a randomly selected detached leaf and from a whole plant of each species after three days and again after three weeks. The leaf segments were cleared, following the methods of Bruzzese and Hasan (1983); and examined for rust infection.

Throughout the two years of pathogenicity experiments with the rust pathogen, plants of all the Lactuceae species tested in the experiments were placed in close proximity to rust infected *H. pilosella* plants.

III.8.1.c Results

No rust sori were formed on any of the non-target species. The control rosettes and leaves of New Zealand *H. pilosella* produced sori in all experiments, though on less than 100% of the detached leaves and whole rosettes (Table 3.14).

No signs of resistance were seen with the naked eye on the non-target species. Leaf clearing showed that urediniospores had germinated on leaf surfaces of all the species. Appresoria were observed on the stomata of *H. lepidulum*, *Sonchus kirkii* and *Embergeria grandifolia*; but there was no evidence of intercellular mycelial growth.

At no stage were rust pustules observed on any of the non-target species growing in the glasshouse next to rust infected *H. pilosella* plants. Self spread of the rust was commonly noted on *H. pilosella* plants during this time.

III.8.1.d Discussion

The results supported the description (Wilson and Henderson, 1966) of the rust pathogen, *Puccinia hieracii* var. *piloselloidarum*, as specific to the subgenus *Pilosella*. Most New Zealand native species of the Lactuceae were included in the host specificity testing, exceptions being *Picris hieracioides* L. and *Taraxacum magellanicum*. Shultz-Bip. A further seven adventive or economic genera of the Lactuceae were also tested. There was no evidence of infection on any species outside the subgenus *Pilosella*.

The use of detached leaves for host specificity testing was included because of the generally higher infectivity levels achieved on *H. pilosella* in previous experiments, and by Scott (pers. comm. 1995a), compared to the infectivity levels on whole plants, in terms of number of replicates infected. Thus, while the use of detached leaves may have given optimistic results for screening of isolates for effectiveness on *H. pilosella*, there was perhaps, extra conservatism in safety testing of non-target hosts. Watson (1985) stated that host ranges of *Puccinia* species have been artificially expanded within controlled environment containment facilities, even on whole plants.

Table 3.14. Host range testing of *P. hieracii* var. *piloselloidarum*. Values are the percentage of sample plants producing sori.

Host	Group						
	I	I	I	I	II	II	II
Rust Inoculum	BRIT12	BRIT32	BRIT12	BRIT32	ÉIRE14	ÉIRE4	ÉIRE14
No. times tested	20	5	20	5	10	5	5
Rosette or detached leaves	Leaf	Leaf	Rosette	Rosette	Leaf	Rosette[a]	Rosette[a]
(a) *Hieracium pilosella*							
ex HpilHOs	84	88	64	76			
ex HpilTWz					90	40	60
(b) New Zealand native Lactuceae							
Embergeria grandifolia	0	0	0	0	0	0	0
Kirkianella novae-zelandiae	0	0	0	0	0	0	0
Microseris scapigera	0	0	0	0	0	0	0
Sonchus kirkii	0	0	0	0	0	0	0
(c) Adventive Lactuceae							
Crepis capillaris	0	0	0	0	0	0	0
Hieracium lepidulum	0	0	0	0	0	0	0
Hieracium murorum	0	0	0	0			
Hypochoeris glabra	0	0	0	0	0	0	0
Hypochoeris radicata	0	0	0	0	0	0	0
Taraxacum officinale	0	0	0	0	0	0	0
(d) Economic Lactuceae							
Cichorium intybus					0	0	0
Lactuca sativa cv. Cos					0	0	0
cv. Red Sails					0	0	0
Tragopogon porrifolius					0	0	0
(e) Economic Asteraceae							
Achillea millefolium L.					0	0	0
Helianthus annuus L.					0	0	0
(e) Other families							
Dactylis glomerata L.					0	0	0
Lolium perenne L.					0	0	0
Lotus corniculatus L.					0	0	0
Lotus pedunculatus Cav.					0	0	0
Phleum pratense L.					0	0	0
Sanguisorba minor Scop.					0	0	0

[a] Where the plants do not form rosettes (e.g. grasses, this refers to "seedling")

For efficiency and a potential measure of the level of host specificity, the testing of potential biological control agents of weeds can concentrate first on the most closely related species and then outwards on progressively less related species. This is the centrifugal testing method of Wapshere (1974). Wapshere (1974) suggested the inclusion of plant species potentially at risk in terms of 1) being recorded as a host of the proposed control agent; 2) being related to the target host; 3) possessing similar secondary metabolites or morphological similarities to the target host; 4) never having been exposed to the agent; 5) having limited records of its natural enemies; or 5) being attacked by related organisms. Weidemann (1991) argued that the phylogenetic method of selecting test species is most precise if a pathogen is highly host specific and is well characterised as such in the literature, as compared to pathogens that might infect species of several families. *Puccinia hieracii* var. *piloselloidarum* was expected to be highly specific, in fact to the subgenus *Pilosella* and testing within the Lactuceae may be sufficient. Nevertheless, with the aim of eventual introduction of the rust to New Zealand as a biological control agent, testing of host specificity remained a principle component of research. Several economic species from other plant tribes and families were therefore tested for susceptibility.

In several biological control of weeds programmes, researchers have suggested that strict host specificity may not always be necessary. Bruzzesse and Hasan (1986) found that strains of blackberry rust *Phragmidium violaceum* proposed for introduction into Australia to control *Rubus fruticosus* L. agg. were able to infect some other blackberry species though the main commercial blackberries were immune to highly resistant. They argued that if there was interest in growing species which were rust susceptible, then fungicidal sprays were available to control the rust. Jacky (1899) reported that *Puccinia carduorum* Jacky was confined to the genus *Carduus* L., but Politis *et al.* (1984) were able to infect seedlings of *Cirsium* spp. and *Cynara cardunculus* L. (globe artichoke) with the fungus. Infection was much less aggressive or absent on older plants of the non-target genera and Politis *et al.* (1984) suggested that the rust fungus could be employed as a biological control agent of *Carduus nutans* L. (musk thistle) in the USA because the susceptibility of globe artichoke was limited (in terms of rate of pustule

development, pustule size and number of sori) and there were no reports from Europe of *P. carduorum* infecting globe artichoke in the field. Bruckart (1989) considered *P. jaceae* to be suitable as a biological control agent of yellow star thistle (*Centaurea solstitialis* L.) because infection on a few non-target species was limited and only occurred under very favourable inoculation conditions.

P. hieracii var. *piloselloidarum* exhibits strict host specificity and is not recorded on any non-target host. Other varieties of *P. hieracii* have been recorded on a range of hosts. These hosts are almost entirely within the Lactuceae though *P. hieracii* var. *hieracii* is also recorded on *Centaurea* of the tribe Cardueae (Parmelee and Savile, 1981). It is likely that within *P. hieracii* var. *hieracii* there are many forms that differ in host range such that cross infection of different species will often not occur. The typical specificity of members of this species can in part be seen by the absence of any rust records on any *Hieracium* spp. in New Zealand despite the common occurrence of two varieties of *P. hieracii* (var. *hieracii* and var. *hypochoeridis*) infecting related genera such as *Taraxacum* and *Hypochoeris*.

III.9 EFFECT OF RUST ON *H. PILOSELLA* GROWTH

III.9.1.a Introduction

As detailed in Section I.2.2.b, it is desirable to provide a measure of the efficiency of a biological control agent on the weed problem. This allows a decision to be made on whether the agent is worth investigating further. *P. hieracii* var. *piloselloidarum* has been shown to be able to infect a number of species of *Hieracium* and a range of genotypes of *H. pilosella*. Rust was generally common in the field and at times appears to have a serious effect on host patches. This experiment measured the effect of rust infection on plant biomass under controlled conditions to see whether this effect is significant.

III.9.1.b Materials and methods

Thirty two *H. pilosella* seedlings were grown from the seed from one capitulum of locality HpilHAs. The seedlings were assumed to be isogenic; the reliability of this assumption is discussed in Section VII.1.4. Seeds were germinated on water agar (0.95%) and transferred to individual pots (7cm x 7cm wide, 8cm deep of one part John Innes No.2 with one part sphagnum peat). Watering was from below with the use of a tray. One rosette was examined cytologically and was pentaploid. The same rosette was sampled for isozyme electrophoresis (rosette code 'HpilHAs5X(i)') and found to have the same pattern for the enzymes GOT and 6-PGD as the majority of tested New Zealand *H. pilosella* (see Section VII.2.6.c). When the seedlings were six weeks old, twenty uniform rosettes were selected for the growth rate experiment on the basis of very similar sizes and numbers of leaves (six to seven fully opened leaves).

Rust inoculum was from one isolate (ÉIRE14). Urediniospores of this strain cultured on pentaploid New Zealand *H. pilosella* host plants (ex HpilHAs) were collected by the use of a vacuum apparatus. Sufficient inoculum was present for the inoculation of 11 of the twenty selected rosettes. A moist cotton bud was used to pick up urediniospores and, after further moistening, was brushed on leaf surfaces to inoculate the rosettes. Inoculation was made using moistened cotton buds and covered all open leaves of 11 randomly chosen rosettes. Control plants (9) were rubbed with moist cotton buds lacking rust inoculum. All rosettes were immediately placed in a moist chamber, sprayed with distilled water, and kept in high humidity for 16h at 20°C with continuous dark. At the end of this period, the chamber cover was removed allowing in light and eventual evaporation of excess water from the leaf surfaces. Rosettes remained in the chamber for a further 24h with 8h light and 12h dark; then transferred to heated glasshouse conditions (25°C maximum, natural light); and placed in random positions in a tray and watering was again from the base.

The subsequent presence of rust sori was noted. After seven weeks the rosettes were harvested, divided into shoots (stems and leaves) and roots (short rhizome and root system); weighed fresh; dried for 40h at 90°C; and weighed.

The experiment was conducted from January through to the end of February (with day length ca. 9 hours) at the CSIRO Institute of Biological Control, Montpellier, France.

III.9.1.c Results

Inoculation was only partially successful with just four of the 11 inoculated rosettes developing moderate infection on most of the opened leaves of each rosette. The four infected plants had an average of 3.5% of the surface of all opened leaves covered by small uredinia. The remaining six inoculated rosettes and all the control rosettes failed to develop any sori within four weeks of inoculation. Secondary infection (i.e. after the primary inoculation) by the rust was uncommon on the infected plants due to the lack of humidity on the leaf surfaces; the only extra infection noted was on some senescing leaves near the soil surface on all of the infected rosettes and on a portion of one senescing leaf of one of the apparently unsuccessfully inoculated rosettes. Neither inoculated nor control rosettes had initiated flowering or stolons. The average values of harvested fresh and dry weights are presented in Table 3.15.

Table 3.15. The effect of rust infection on *H. pilosella* shoot and root biomass.

Treatment	Fresh Weight (g)		Dry Weight (g)		Root:Shoot
	Root	Shoot	Root	Shoot	Ratio
Inoculated (sori produced)	1.58^{a}	1.15^{a}	0.226^{a}	0.277^{a}	0.82^{a}
Inoculated (no sori)	2.09^{ab}	1.50^{b}	0.356^{b}	0.347^{ab}	1.03^{a}
Uninoculated (control)	2.13^{b}	1.72^{b}	0.388^{b}	0.416^{b}	0.93^{a}

Treatments with ^{a} are statistically significantly different ($p<0.05$) from treatments with ^{b} in a column. Treatments with ^{ab} are intermediate. Significance was derived from lsd values.

A general linear model analysis demonstrated that differences in shoot and root dry weights between successfully inoculated and control rosettes were highly significant ($p<0.01$). The differences in fresh weight were significant for roots ($p<0.05$) and highly significant for shoots ($p<0.01$). The average weights of the unsuccessfully inoculated treatments were all less than those of the control, though not statistically

significant. However, the combined dry weight averages for successful and unsuccessful inoculation treatments were significantly less than those of the control according to a t-test; root weights were less ($p<0.05$) and shoot weights were highly significantly less ($p<0.005$).

Shoot and root dry weights of successfully inoculated rosettes were 67% and 58% respectively of the weight of control rosettes. When all inoculated rosettes were considered together regardless of the presence of infection, the values were 77% (shoot dry weight) and 79% (root dry weight) of the control rosettes. Root to shoot ratios were generally lower in the successfully inoculated plants but the differences were not statistically significant.

III.9.1.d Discussion

The lower weight of infected plants at the end of the seven week period indicated a significant effect of the rust on plant growth. Inoculation with rust resulted in 22 to 33% reduction in shoot dry weight and 21 to 42% reduction in root dry weight. The experiment involved the selection of genetically and visually similar rosettes, and uniform growth conditions for each rosette to minimise variation not due to treatment. This estimate of reduced plant growth compared favourably with an estimate of 2.7% per log(sori+1) made by Scott (pers. comm. 1995a) for the effect of British rust isolates on growth of *H. pilosella* shoots over six weeks.

Several international weed biological control programmes involving rust pathogens have included an assessment of the effect of the pathogen on the growth of the host weeds. Parker *et al.*(1994) reported that *Puccinia abrupta* var. *partheniicola* (Parm.) Jackson caused 53% reduction in the above ground dry matter relative to controls of *Parthenium hysterophorus* L. in glasshouse experiments. The value was based on ten plants inoculated every three to four days from the age of 13 weeks. In the second season of a field experiment involving *Chondrilla juncea*, Emge *et al.* (1981) reported a biomass reduction of 89% (relative to control plants) caused by *Puccinia chondrillina*. Alber *et al.* (1985) conducted an experiment on *Senecio jacobaea* L.

with the rust pathogen, *Puccinia expansa* Link. whereby *S. jacobaea* was inoculated fortnightly from the age of five to six weeks, resulting in an above ground biomass (dry weight) reduction (relative to controls) of 50% at the age of 78 to 79 days and 62% at 108 to 119 days. Hasan (1974a) reported that *Puccinia xanthii* Schw. caused a 37% reduction in above ground dry weight of *Xanthium strumarium* L. in field experiments. *Centaurea solstitialis* L. exhibited dry weight reductions of 40% in roots and 50% in shoots caused by *Puccinia jaceae* Otth.; measured ten weeks from seed after inoculation four times per week from four till seven weeks of age (Bennett *et al.*, 1991).

The present work did not measure the possible effect of *Hieracium* rust on floral and stolon production i.e. the reproductive potential of the rosettes. This was due to time constraints and the British quarantine requirements that New Zealand *Hieracium* be not allowed to flower. The *Parthenium hysterophorus* rust for instance, caused ten fold reduction in the production of flowers, hastened leaf senescence and shortened the lifespan of infected hosts (Parker *et al.*, 1994). *Puccinia chondrillina* has also been reported to significantly reduce flower production, viability of seed and plant size (Adams and Line, 1984); kill young seedlings, often prevent older plants from sprouting new rosettes after overwintering (Hasan and Wapshere, 1973); decrease competitive ability (Burdon *et al.*, 1984); and has been extremely successful in Australia in virtually eliminating the weed problem of one genotype of *Chondrilla juncea*. In the second season after one artificial inoculation of *P. chondrillina*, there was an 89% reduction in the flowering stalk dry weight of *C. juncea*, 94% fewer seeds with 30% less germination and 24% lower seed weight. In an evaluation of the potential of *Rumex* rust for the control of curly dock, Inman (1971) reported that only 43 % of target plants resumed growth in the season following inoculation of field plots. Plots kept rust free with fungicide had 95% regrowth of curly dock.

Some rust fungi are systemic (spreading throughout host tissue), for example, *Puccinia punctiformis* which often causes *Cirsium arvense* rosettes to fail to survive a season (Forsyth and Watson, 1985). *P. hieracii* varieties and the rusts mentioned in the previous paragraphs produce localised infections only. The effect of rust infection

on plant parts demonstrates the ability of localised rust infection to reduce the growth of other plant parts. Plants may put relatively more resources into shoot growth than root growth when they are infected with rust; Ahmad *et al.* (1982) found that after brown rust sori production on inoculated first leaves of young barley plants (four leaves in total), the root:shoot ratio was very significantly reduced.

A major feature encountered with the rust pathogen was noted; only four of the eleven inoculated rosettes gave rise to rust sori. These four rosettes shared even infections over most of the fully opened leaves, excluding the two youngest inoculated leaves and several of the older leaves. Since the rosettes were highly likely to be isogenic, rust infection may have been due to environmental conditions.

Chapter IV RUST HOST/PATHOGEN RELATIONSHIPS - FIELD STUDIES

IV.1 SEASONAL FLUCTUATIONS OF RUST ON *H. PILOSELLA*

IV.1.1.a Introduction

Large seasonal fluctuations in levels of rust sori were noted in field surveys. In order to demonstrate the scale of these fluctuations, intensive observations were made on ten patches of *H. pilosella* in various localities in the Holyrood Park area of Edinburgh. This section records the levels of active rust sori on each patch while the levels of sori invaded by secondary pathogens and those with an amorphous covering are recorded in Sections IV.3 and IV.4 respectively.

IV.1.1.b Methods

Ten patches of *H. pilosella* were selected from Holyrood Park. All patches were in sites exposed to extremes of seasonal conditions, on slopes facing southwest to southeast (sunny) to approximate conditions in the New Zealand high country. Rainfall is around 700 mm per annum in the Edinburgh area as compared to 600-650 mm in some of the worst *H. pilosella* affected areas of the New Zealand Mackenzie country. The chosen site localities at Holyrood Park were known to experience dry conditions in summer. Ten surveys were made over the course of one year. For each survey, 50 rosettes were randomly selected in each patch and number of sporulating sori were assessed on each of these rosettes to an accuracy of at least ten sori.

IV.1.1.c Results

The majority of sori were uredinia producing urediniospores, but teliospores were noted in sori during late autumn and winter. No pycnia or aecia were noted on the rosettes investigated; these stages were very seldom observed in the Holyrood Park area. The changes in numbers of sporulating sori per plant over the year in each of the ten patches are shown in Table 4.1. A plant was defined as a rosette with a developed root system.

Highly significant ($p=0.0001$) differences in the numbers of sporulating sori were evident between patches and between dates of observation according to analysis of variance. At each sampling, analysis of variance showed the difference between mean numbers of sporulating sori of localities to be highly significant ($p<0.005$). The interaction of date and patch was also highly significant indicating differences between patches in their rust infection level changes (based on records of sporulating sori) over the seasons. Significance values are derived from the Duncan's Multiple Range Test.

Uredinia are present in very early spring producing viable urediniospores. High levels of infection were common on patches by mid-spring. Levels of infection generally dropped off after mid-summer, particularly on exposed rosettes. High levels of sporulating sori were still noted on rosettes surrounded by tall turf.

Uredinia are present throughout autumn. Mid autumn saw a secondary peak of infection in two patches, but levels were low compared to the spring/early summer peaks. Levels of infection dropped off again in the winter. At many sites however, it was observed that high levels of infectivity occurred in the very early spring at many sites with a rapid (sometimes over one week) response to brief warm conditions. Uredinia and telia were observed to survive and be sources of inoculum during winter and into spring. These sori apparently only remained open in relatively mild microclimates where plant tissue was still green. In many cases a covering, similar to that seen in summer, was observed to form on the sori.

Table 4.1. Mean numbers of sporulating rust sori per plant, in ten patches over one year.

Locality	Date of Observation										
	Early Mar. 1993	Mid June 1993	Early July 1993	Late July 1993	Late Aug. 1993	Mid Sept. 1993	Early Oct. 1993	Mid Nov. 1993	Early Jan. 1994	Early Mar. 1994	**Overall**[a]
Innocent Railwayline (i)	15bc	64b	43b	21b	7.6bc	0.04c	2.9bc	17a	0.04	3.0ab	**17C**
Innocent Railwayline (ii)	2.1c	17d	12c	3.5def	0.3d	3.1abc	5.1ab	1.4b	0	3.1ab	**4.4E**
Samson's Ribs	2.2c	150a	23bc	14bc	9.7ab	2.0bc	1.3c	13a	0.4	0.02c	**21B**
The Hawse	43a	2.3d	1.1c	0.8ef	0.02d	0.02c	0.3c	0.3b	0	3.9a	**5.1E**
South Quarry M	11bc	3.6d	1.2c	0.02f	0.04d	0.3bc	0.6c	1.0b	0.6	0.5bc	**1.9E**
Cat Nick Slope	7.9c	57bc	180a	21b	16a	6.4a	8.0a	3.3b	0b	0.5bc	**28A**
Galloping Glen N	8.5c	11d	1.0c	8.1cde	1.1cd	0.2c	0.7c	0.4b	0b	1.0bc	**3.1E**
Galloping Glen S	24b	11d	10c	14bc	0.3d	4.0ab	0.2c	0b	0b	0.02c	**6.3E**
Girnal Crag	55a	33cd	15c	9.3cd	12ab	1.4bc	1.1c	1.1b	0.1b	1.6abc	**13D**
Dunsapie Crag	3.6c	22d	46b	34a	11ab	3.7abc	3.1bc	4.3b	0b	0.02c	**13D**
Total[a]	**17C**	**36A**	**32B**	**13D**	**5.8E**	**2.1EF**	**2.3EF**	**4.2EF**	**0.1F**	**1.3EF**	

[a] Significant differences between mean numbers of sori per plant in each date and each locality are indicated with capital letters at the end of each column and each row respectively. Values with the same capital letter are not statistically significantly different (p=0.05). Significant (p=0.05) differences between means of localities within each date are indicated with lower case letters, values with the same letter are not significantly different. The small case letters are not valid for comparisons between dates.

A comparison between the ranking of patches in March 1993 with March 1994 was made with Spearman's Rank Correlation. The correlation was not statistically significant indicating that those patches with higher levels of actively sporulating rust sori in March 1993 did not necessarily have higher levels than other patches in 1994.

The levels of covered sori are presented separately in Section IV.4.1.c and levels of sori surrounded by necrotic lesions are presented in Section IV.3.1.c(i).

IV.1.1.d Discussion

The results demonstrate large fluctuations in the numbers of sporulating sori with time. Peak production of uredinia was in spring to early summer, with a secondary peak for some patches in autumn. Levels of sporulating uredinia during the late summer were relatively low; and very low on most exposed rosettes. The presence of actively sporulating rust sori on rosettes sheltered by surrounding grass may have been due directly to less moisture stress or indirectly to a difference in host condition. Shading could have increased potential for infection by causing longer periods of moist conditions on hosts. Rosettes surrounded by tall grass were often observed to have delayed and reduced flowering which may affect susceptibility to rust. In field collections the frequency of rust on non-flowering rosettes was generally higher than that on flowering rosettes at the same stage of the season.

Rust overwintered as sori and also as mycelium within leaf tissue. Teliospores may also have overwintered and the occasional presence of pycnia in the spring on some patches was evidence of sexual activity.

There are several reasons for the demise of sori over winter. Freezing damage appears to be very prevalent in sori (Fig 3.18). Secondary pathogens can also invade sori and cause necrosis of rust sori and host tissue (see Fig 4.1). The senescence of older leaves occurs through cold damage and also old age. These older leaves often host a large proportion of the rosette's sori. Furthermore whole rosettes may die due to environmental conditions or due to setting seed (*H. pilosella* is a monocarpic

perennial). In colder sites at Pitlochry and Aviemore, of the Scottish Highlands, no sori were observed to survive open over the winter periods of 1992/1993 and 1993/1994. In both these areas the rust appeared to survive within leaf tissue presumably as mycelium or covered uredinia.

The seasonal fluctuations of *Puccinia chondrillina* on *Chondrilla juncea* were reported by Hasan and Wapshere (1973). Infection and spread of *P. chondrillina* was limited during winter followed by a vigorous formation of uredinia in early spring. Hasan and Wapshere (1973) stated that infections occurred throughout the year. The phenomena of low winter presence with survival of the uredinial stage, and early spring increase in sporulation were similar to the observations of *P. hieracii* var. *piloselloidarum*.

The large fluctuations in sori levels over the period of the survey varied between patches with a general trend of a peak in spring and a smaller peak again in autumn. The lack of correlation between ranking of patches in March 1993 and March 1994 indicated the differences between patches in their pattern of fluctuation, though in March 1994 values were generally lower than in March 1993.

IV.2 FACTORS AFFECTING THE SEVERITY OF RUST ON *H. PILOSELLA*.

Field observations indicated a variation in the severity of rust disease, even within a site. In order to elucidate the main factors affecting the level of disease in *H. pilosella* patches, a survey was conducted of several hundred patches of the host in the Holyrood Park area, Edinburgh. The level of rust infection on each patch and potential factors influencing the infection severity were recorded. Collections of rosettes from patches not exhibiting rust symptoms were tested for susceptibility to rust urediniospores taken from nearby rust infestations.

IV.2.1.a INTRODUCTION

IV.2.1.a(i) The field site

The Holyrood Park area consists of a series of hills, the highest of which is Arthurs Seat with a top called Lion's Head at 251m. The area is of volcanic origin. A major feature are the Salisbury Crags on the western side of the park. The Crags are a long stretch of rock face curving around the top of a hill the highest point of which is 175m. Below the rockfaces are large slopes with aspects from southwest to northwest. The human influence has been great on the Park area from previous cultivation and quarrying to the present, solely recreational, use. There are two tarsealed roads, several sealed and gravel tracks and a network of foot tracks. Rabbit presence is conspicuous in both sighting of rabbits and sign. The vegetation is mostly grassland. The majority of the grass cover is a high turf most of the year. On the tops of hills and some exposed areas facing south to southwest the turf is generally low. There is very little tree cover, though some slopes have a partial covering of gorse (*Ulex europaeus* L.). Most areas of the park have a degree of rocky terrain.

IV.2.1.a(ii) Presence of *Hieracium* and pathogens

There are several species of *Euhieracia* in the area including *H. murorum* agg. and *H. sylvaticum* agg. The *Euhieracia* were commonly infected by rust (*Puccinia hieracii* var. *hieracii*) and powdery mildew (*Erysiphe cichoraearum*). *H. pilosella* was the predominant species of subgenus *Pilosella* present.

H. pilosella was found throughout the Park particularly on dry slopes, in low turf with good drainage, by tracksides, and amongst outcrops of rocks. Patches varied in size from over two metre diameters on an exposed part of Dunsapie Crag (ca. 130m) to just a few rosettes amongst rock outcrops. On the slopes of the Salisbury Crags and Arthur's Seat with aspects from southwest to west *H. pilosella* was very common. *H. pilosella* was also very common on either side of the northwestern extent of an abandoned railway line (Innocent Railwayline). *Hieracium* spp. were uncommon on the flat mown areas of the Park. The rust fungus *Puccinia hieracii* var. *piloselloidarum* was common on members of *Pilosella* in the southern and western areas of the Park and powdery mildew was locally common within the same areas. The rust was uncommon in the northern areas where the *H. pilosella* was less common and grass cover was taller.

IV.2.1.b Methods

IV.2.1.b(i) Survey

236 patches of *H. pilosella* in the Holyrood Park area were surveyed over a three day period in late summer (September 2-4), 1993. These were all the patches that were found during an intensive survey throughout most of the Park. The presence of rust symptoms in each patch was recorded in terms of percent of plants infected (calculated from random sampling of twenty rosettes), rust severity in terms of actively sporulating sori per leaf (on a scale of 0 to 5 as in Table 4.2) and estimations of the average number, per rosette leaf, of sori no longer sporulating, and of sori hosting visible secondary pathogen infections.

Table 4.2. Scale of rust severity. (Based on number of actively sporulating sori).

SCALE	SYMPTOMS
0	none present
1	<2 sori per leaf
2	3-5 sori per leaf
3	6-10 sori per leaf
4	11-15 sori per leaf
5	>15 sori per leaf

A code was given to each site location of the park sampled to allow comparison between groups of patches. Site factors recorded for each patch were aspect (rounded to 45° angles and split into north-south and east-west components) and slope (estimated to nearest 5°), distance to nearest *H. pilosella* patch (1, 3.5, 7 or 40m), patch size (diameter in metres) and density of rosettes (estimated to the nearest 500 rosettes m^{-2}), presence of moisture in soil, stony soil, shallow soil and high soil organic matter (all yes or no), powdery mildew infection (estimated to the nearest 5% cover over the whole patch), and percentage of bare ground, rock and tall grass (all estimated to nearest 5%).

The data were analysed with Correspondence Analysis and stepwise Multiple Regression to identify environmental factors affecting presence and severity of rust.

IV.2.1.b(ii) Genetic susceptibility

Rosettes of 12 patches lacking rust symptoms were transplanted to the glasshouse. After three weeks the rosettes were examined for the presence of sori from latent infections and naturally or accidentally transferred urediniospores. When no rosettes from a certain patch exhibited rust infection, rosettes were tested for susceptibility to a rust isolate collected near to the location of each patch sampled. Five individually potted rosettes of each patch were inoculated using moist cotton buds according to the methods in Section II.2.4.b. Ten leaves were excised from remaining rosettes of each location and inoculated according to the method in Section II.3.1.a. The presence of sori was recorded four weeks after inoculation. In addition, six patches from near Aviemore (Scotland) and one from Bush (Mid Lothian, Scotland) were sampled and tested for susceptibility in a similar manner. The Aviemore rosettes and detached leaves were inoculated with various BritNU isolates (from near Aviemore) and those from Bush were inoculated with isolate BritIR(i) (from Edinburgh).

IV.2.1.c Results

IV.2.1.c(i) Survey

Infection levels varied from all rosettes in a patch being infected to no visible infection at all. An average of 78% of rosettes on the surveyed patches were infected. Five percent of patches had no visible infection on rosettes and 46% of patches had all sampled rosettes infected. Rust severity varied from 0 to 2.5 with an average of 0.36 out of a maximum possible score of 5. Results of covered sori are presented in Section IV.4.1.c and those of sori invaded by secondary pathogens in Section IV.3.1.c(i).

The site locations into which patches were grouped are shown in Table 4.3 with their respective mean values of percentage rosettes infected and rust severity. Multiple regression analysis found percentage rust ($\log_{10}$(percentage plants infected + 1^{-10})) was significantly related to two site factors as well as the site location ($p<0.001$). Distance to the nearest adjacent patch ($p<0.001$) accounted for 13% of the variation on its own; those patches nearer to other patches had a generally higher percentage of infected rosettes. Patches with higher amounts of surrounding rock had lower percentages of plants infected ($p=0.026$). A model including site location, distance to nearest patch, and amount of surrounding rock accounted for 68% of the variation in percentage rust.

Rust severity (square root transformed) was positively correlated with the presence of stony soil ($p=0.003$), explaining 15% of variation in severity. The severity of rust was significantly related to the site location ($p<0.001$). With site location incorporated in the model, stony soil ($p=0.002$) and high soil organic matter content ($p=0.015$) were both positively related to rust severity; this model accounted for 14.9% of variation.

Multiple correlation analysis allowed the relationships between measured site factors to be investigated. The only highly significant correlation seen was between patch diameter and slope ($p<0.05$) such that patches were generally larger on steeper slopes. The relationship was likely to be due to the generally lower competition offered by other plants on the drier, slope areas. There was no significant correlation between any two of the site factors used in the above models.

Table 4.3. Abundance of rust on *H. pilosella* at Holyrood Park sites.

Site Location	% rosettes infected	mean rust severity[a]	no. of observations
Haggis Knowe	0	0	6
Woodpecker Gully	0	0	2
Samsons Ribs	20	0.15	15
Radical Road N	22	0.08	2
Dunsapie Crag	22	0.15	14
Radical Road S	23	0.05	6
The Hawse	23	0.1	33
Girnal Crag	23	0.12	11
South Quarry A	24	0.09	23
Cat Nick Slope	24	0.17	11
Long Quarry A	25	0.09	5
South Quarry S	25	0.12	14
South Quarry N	26	0.02	2
Galloping Glen S	26	0.13	15
Innocent Railwayline	27	0.15	35
South Quarry Slope	28	0.11	1
Galloping Glen N	28	0.14	13
South Quarry M	29	0.13	5
Salisbury Crags Low	30	0.05	6
Radical Road Slope	30	0.11	15
Long Quarry Slope	30	0.18	1
Gutted Haddy	30	0.23	1

Site codes are taken from names of areas in the Park on the Scottish National Trust map.
[a] Rust severity was measured according to the scale in Table 4.2

IV.2.1.c(ii) Genetic susceptibility

Plants from all patches tested demonstrated susceptibility to infection by rust (Table 4.4). Infection was recorded on an average of 39% of detached leaves and 44% of whole plants. Rust sori were present on rosettes of four of the patches (indicated by superscript 'b' in Table 4.4) without artificial inoculation. These included two rosettes of Inverdruie(i) (near Aviemore) which exhibited rust sori five days after collection. No sori on detached leaves displayed any signs of hypersensitivity (chlorosis or necrosis). Three weeks after sori eruption, some sori on older leaves of most Aviemore rosettes exhibited chlorotic regions around them. These sori were relatively large and numerous secondary infections were produced.

IV.2.1.d Discussion

IV.2.1.d(i) Rosette distribution and infection

The most significant factor affecting the percentage of rust infected rosettes was the distance to the nearest patch. Because of great abundance of *H. pilosella* infestation in many areas of the New Zealand high country, distance between patches would generally not be a limiting factor to the spread of *Hieracium* rust. The reasons for significance of the distance factor in the Holyrood Park area are likely to include the proximity of rust inoculum to allow an earlier and faster rate of epidemic spread of rust over the season from patch to patch.

No correlation was noted between density of rosettes within a patch and the percentage and severity of rust infection. Apparently the differences in proximity of rust inoculum between the individual rosettes in a patch was insufficient to lead to significant differences in rust levels due to *H. pilosella* patch density. The density of plants is often a significant factor with other host plants and their pathogens (Burdon and Chilvers, 1982). Burdon and Chilvers (1977) found that reduced barley powdery mildew in mixtures of barley and wheat was mainly due to reduction in density of barley with a minor effect of wheat plants trapping spores. They noted that the

Table 4.4. Susceptibility of rosettes from rust free patches to nearby rust isolates.

Host Code	Location	Rust Code	Distance (inoculum to host)	Detached Leaves[a]	Whole Rosettes[a]
Samson's Ribs (i)	Holyrood Park	IR xv	c. 100 m	3 (+,+,++)	2 (+,+)
Samson's Ribs (ii)	Holyrood Park	AS iii	c. 800 m	4 (+,+,++,++)	2 (+,++)
Samson's Ribs (xii)[b]	Holyrood Park	-	-	-	-
C (i)	Holyrood Park	C iii	10 - 30 m	7 (+,+,+,++,++,++,++)	3 (+,+,++)
Samson's Ribs (viii)	Holyrood Park	MB iii	0.1 - 2 m	2 (+,++)	2 (+,+)
Samson's Ribs (ix)	Holyrood Park	MB x	5 - 10 m	4 (+,+,+,++)	1 (+)
Haggis Knowe (i)	Holyrood Park	Q i	c. 800 m	3 (+,+,+)	3 (+,+,+)
Haggis Knowe (ii)	Holyrood Park	Q i	c. 800 m	3 (+,+,+)	1 (++)
Haggis Knowe (iii)	Holyrood Park	Q i	c. 800 m	4 (+,+,+,++)	3 (+,+,+)
Haggis Knowe (iv)	Holyrood Park	Q i	c. 800 m	5 (+,+,+,+,+)	2 (+,++)
Haggis Knowe (v)	Holyrood Park	Q i	c. 800 m	2 (+,1)	1 (+)
Haggis Knowe (vi)	Holyrood Park	Q i	c. 800 m	2 (+,+)	2 (+,+)
Nursery (iii)	Aviemore	NU i	c. 30 m	6 (+,+,+,+,++,++)	4 (+,+,++,++)
Nursery (iv)	Aviemore	NU ii	c. 30 m	4 (+,+,++,++)	2 (+,++)
Nursery (v)	Aviemore	NU iii	c. 30 m	6 (+,+,+,++,++,++)	2 (++,++)
Alcove (i)[b]	Aviemore	-	-	-	-
Inverdruie (i)[b]	Aviemore	-	-	-	-
Inverdruie (iii)[b]	Aviemore	-	-	-	-
Bush	Bush	IR xv	c. 10 km	3 (+,++,++)	3 (+,+,++)

[a] Each infected leaf or rosette was classed as + (= a few sori) or ++ (>10 sori on detached leaves, >20 sori on whole rosettes).

[b] Rosettes collected from these patches exhibited rust symptoms within three weeks after transplanting to the glasshouse without artificial inoculation.

trapping factor may be more important at high non-host densities. Berger (1975) reported higher levels of the pathogen *Cercospora apii* on celery when the hosts were planted at higher densities; the increase in disease was attributed to microclimatic differences, especially increased humidity, rather than diminishing gradients of spore deposition, as the distances involved were small and the inoculum readily wind-disseminated. In grassy areas, an increase in the density of *H. pilosella* could have in fact caused reduced humidity because of the suppression of taller plant species. Burdon and Chilvers (1976) showed that a clumped distribution may not be optimum for the rate of disease spread. Observing epidemics of damping-off disease in cress seedlings, they found that although a higher density within a clump led to a moderate increase in disease spread, overall there was a reduction in infection because of the spatial separation of clumps. This effect was likely to be affected by the distances involved within and between clumps and the nature of pathogen spread (damping-off is soil borne). A similar phenomenon is apparently present with *Hieracium* rust i.e. the level of rust was more affected by the distance between patches than the density of rosettes within patches.

Stony soil and low organic matter, were both shown to be significantly related with reduced rust severity, and are linked to potentially dry soil conditions. No relationship was seen between moist soil qualitatively measured at the time of survey and the percentage or severity of rust, but it is possible that warm and dry weather earlier in the season may have caused the lower levels of rust in patches more prone to dry conditions. The relationship between high rock presence and low percentage rust may also have been due to susceptibility to dry soil conditions. The significance of rock presence was highly influenced by the absence of rust in the Samson Ribs area where the two patches surveyed were both growing in rock outcrops.

IV.2.1.d(ii) Site location

The site location of the patch was the most significant factor in percentage rust infection and rust severity. The non-quantifiable nature of the site classifications made it impossible to correlate sites with environmental variables. Some site

locations were, however, quite distinct, including 'Haggis Knowe' and 'Woodpecker Gully' which were both free of visible rust symptoms. Both these locations were relatively well separated from the other sites where *H. pilosella* was common. Some other site locations were separated from the areas of highest *H. pilosella* abundance (the southwest) but had high levels of rust disease; apparently the numbers and positions of the patches were sufficient to maintain the high rust levels. The site 'Haggis Knowe' also had a reasonable number of patches close together but tall grass at that site may have hindered any spread of rust.

IV.2.1.d(iii) General

Experimental inoculations of rosettes from patches free of rust infections demonstrated that the hosts were genetically susceptible to rust infection given suitable environmental conditions. It remains possible that resistance to rust infection under sub-optimum inoculation conditions is under some genetic control. The development of rust sori on rosettes from Polchar(i) (near Aviemore) within a week after transplanting to glasshouse conditions indicated that the rust infection was probably already latent in the plants during collection as the shortest latent period measured for the rust is six days (with isolate ÉIRE14 on detached leaves). Several similar observations of probable latent infections, in rosettes collected from patches not exhibiting rust infection, have been made during field collection trips.

Carlsson *et al.* (1990) conducted a large scale field study of three host specific fungal plant pathogens, *Uromyces valerianae* (a rust on *Valeriana sambucifolia* Mikan fil.), *Urocystis trientalis* (Berk. & Broome) Lindberg (a smut on *Trientalis europaea* L.), and *Ustilago violacea* (Persoon) Roussel (anther smut disease of *Silene dioica* (L.) Clairv.). The study was based in the Skeppsvik archipelago, a series of islands of various ages. Disease level (% plants infected) was measured for each pathogen/host system and the correlation of this with host density, and age of the host population (estimated from island age) was measured. Carlsson *et al.* (1990) were testing an hypothesis that newly colonising populations of host plants were likely to be more susceptible to epidemics of pathogens than older populations because of homogenous stands of high host plant densities. They noted generally high levels of infection in

the early to intermediate phases of host population development, with levels dropping off in older populations but no statistically significant correlations could be shown between host population densities and any of the diseases.

The present study differed from that of Carlsson *et al.* (1990) in that a larger range of environmental variables were recorded, and other aspects of disease level were also measured. Population age was not a factor that was measured in the present study. Patch diameter may give an estimate of patch age but the estimate would be affected by environmental and competitive factors and it is not certain whether rust affects the rate with which a patch of *H. pilosella* expands. The rosette density within a patch does not appear to differ significantly enough to affect rust disease level. Population age of *H. pilosella* is likely to be correlated in the early to intermediate phase of invasion with an increase in the number of patches in an area and this was the major factor affecting *H. pilosella* rust levels in the present study.

IV.3 SECONDARY PATHOGENS

IV.3.1 Impact of secondary pathogens on rust effect

IV.3.1.a Introduction

Field observations indicated that rust sori on *H. pilosella* were often invaded by secondary pathogens. Observations of invaded sori were incorporated into the field surveys at Holyrood Park. Several fungi were isolated from the invaded sori, identified and kept in permanent culture.

IV.3.1.b Methods

IV.3.1.b(i) Field observation

The early September (late summer) survey of 236 *H. pilosella* patches in Holyrood Park (Section IV.2.1.b(i)), included an estimate of the number of invaded sori per rosette for each patch. Multiple regression analysis was applied to elucidate the environmental factors significantly affecting the presence of invaded sori as according to the methods in Section IV.2.1.b(i). The monitoring of ten patches of *H. pilosella*

over the course of a year in Holyrood Park (Section IV.1) also included the counting of invaded sori on each of the 50 sample rosettes in each patch. The dates of sampling are given in Table 4.5.

IV.3.1.b(ii) Isolation of secondary pathogens

Leaves with secondary pathogen lesions were surface sterilised in a 20% solution of commercial bleach (1% v/v sodium hypochlorite) for 30 seconds. Small sections from the edge of several lesions were cut under sterile conditions and placed on potato dextrose agar medium (PDA) with 25 $\mu g\ ml^{-1}$ anti bacterial streptomycin. Isolated fungi were subcultured on to PDA without streptomycin for identification. Three isolates were identified to genus level according to the key of Clements and Shear (1973). Isolates were further subcultured on to PDA slopes in universal bottles for permanent storage.

IV.3.1.c Results

IV.3.1.c(i) Field observations

Symptoms caused by secondary infection were occasionally severe. In the "Hillside Arthurs Seat" ('Samsons Ribs') site, particularly severe infections caused the necrosis of large amounts of leaf tissue. In some cases, infections close to the base of a leaf caused the senescence of the entire leaf.

In the survey of 236 *H. pilosella* patches in Holyrood Park, 93% of patches were found to have some sori invaded by secondary pathogens. The average number of invaded sori was estimated as 0.7 sori per leaf over every patch sampled, the highest average number of sori per leaf in any patch was 5.5 sori.

In the multiple regression analysis a transformation of $\log_{10}(\text{invaded sori} + 1^{-10})$ was used in all correlations with secondary pathogen presence. The level of secondary pathogens was logically related to the level of rust infection, measured by the percent of rosettes in a patch hosting rust sori, with 28.7% of the variation in secondary pathogens related to this one factor. Greater numbers of sori present would offer an increased number of potential sites for infection. The level of secondary pathogens was also strongly correlated with the area in which the patch was located. The areas that had high levels of secondary infection included sheltered areas as well as exposed areas. Density of the rosettes was significant ($p=0.038$) only when area was not included in the model. Patches with a higher density of rosettes were correlated with higher levels of secondary infection.

Higher levels of secondary pathogens were related to greater proportions of moss in surrounding vegetation with ($p=0.011$) and without ($p=0.025$) area included in the model. The two significant site factors, percentage of plants infected with rust ($p<0.001$) and the proportion of surrounding moss ($p=0.011$), accounted for 16.2% of the variation in secondary pathogen levels. A model comprising site location ($p<0.001$), rust severity ($p<0.001$) and moss level ($p=0.003$) accounted for 27% of variation.

The numbers of invaded sori recorded during the time survey are given in Table 4.5 together with the percentage that these values were of the total number of sori present, including sporulating sori and covered sori. High proportions of sori were invaded from early spring until early summer. In late summer to early autumn, proportions were again high. Senescence of older leaves was common during early summer, resulting in the loss of sori including those invaded by secondary pathogens.

IV.3.1.c(ii) Laboratory work

Isolations were made of 16 fungi from necrotic lesions surrounding invaded rust sori. These included three isolates identified to the genus level, *Botrytis* Pers. ex Fr., *Phoma* Sacc. and *Cladosporium* Link ex Fr. An attempt to inoculate conidia of the *Botrytis* sp. onto healthy rust sori on glasshouse plants was unsuccessful.

Scanning electron microscopy (Fig 4.1) was used to demonstrate the necrotic state of tissue around an invaded uredinium and the lack of urediniospore production from the sorus. Hyperparasites were also in evidence. Fig 4.2 shows the hyphae of an unidentified hyperparasite on a uredinium.

IV.3.1.d Discussion

Secondary pathogens were common in the Holyrood Park area and were also noted during field surveys throughout Europe. Spring and autumn are peak periods of secondary infection, probably due to the greater availability of moisture for secondary pathogen growth and of rust sori for points of infection. Symptoms of rust infection can be greatly increased by secondary pathogen invasion. The consequent destruction of uredinia and urediniospores may limit the production of more sporulating uredinia but in many of the patches where secondary pathogen invasion was high, sporulating rust levels were also very high.

Table 4.5. Seasonal presence of secondary pathogen invasion in rust sori on *H. pilosella* at Holyrood Park.

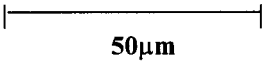
Patch		Mid June 1993	Early July 1993	Late July 1993	Late Aug. 1993	Mid Sept. 1993	Mid Nov. 1993	Early Jan. 1994	Early Mar. 1994
Innocent Railwayline (i)	no. sori invaded	30.2	38.3	0	22.3	0.7	0.2	0	9.5
	% of total	**31**	**47**	**0**	**47**	**24**	**1**	**0**	**63**
Innocent Railwayline (ii)	no. sori invaded	14.5	29	0	5.3	11.9	0.1	0	15
	% of total	**39**	**65**	**0**	**16**	**58**	**2**	**0**	**75**
Samson's Ribs	no. sori invaded	28.3	52.6	0	31.5	12.6	0.3	0	17.1
	% of total	**16**	**69**	**0**	**75**	**70**	**2**	**0**	**77**
The Hawse	no. sori invaded	2.1	6.7	0	0.1	1.3	0	0	0.6
	% of total	**39**	**47**	**0**	**10**	**36**	**0**	**0**	**11**
South Quarry M	no. sori invaded	1.3	5.2	0	4.7	4.7	0	0	0.8
	% of total	**14**	**20**	**0**	**40**	**41**	**0**	**0**	**5**
Cat Nick Slope	no. sori invaded	0	42.2	0	24.2	15.2	3.5	0	0.9
	% of total	**0**	**19**	**0**	**58**	**60**	**51**	**0**	**35**
Galloping Glen N	no. sori invaded	0	13.7	1.3	4.2	5.3	0.3	0	2.1
	% of total	**0**	**64**	**12**	**19**	**52**	**25**	**0**[a]	**20**
Galloping Glen S	no. sori invaded	0.4	3.7	0	2.2	14.1	0	0	0.8
	% of total	**4**	**19**	**0**	**14**	**69**	**0**[a]	**0**[a]	**7**
Girnal Crag	no. sori invaded	4.8	22.6	0	11.2	6.5	0.8	0	0.2
	% of total	**13**	**60**	**0**	**38**	**56**	**42**	**0**	**2**
Dunsapie Crag	no. sori invaded	8.3	12.3	0	9.3	7.5	0.1	0	0
	% of total	**27**	**21**	**0**	**45**	**66**	**2**	**0**	**0**
Overall	no. sori invaded	90	230	1.3	120	80	5.3	0	4.7
	% of total	**19**	**38**	**1**	**43**	**59**	**11**	**0**	**8**

[a] There were no active or covered sori evident on the sampled rosettes during the marked sample times at these patches.

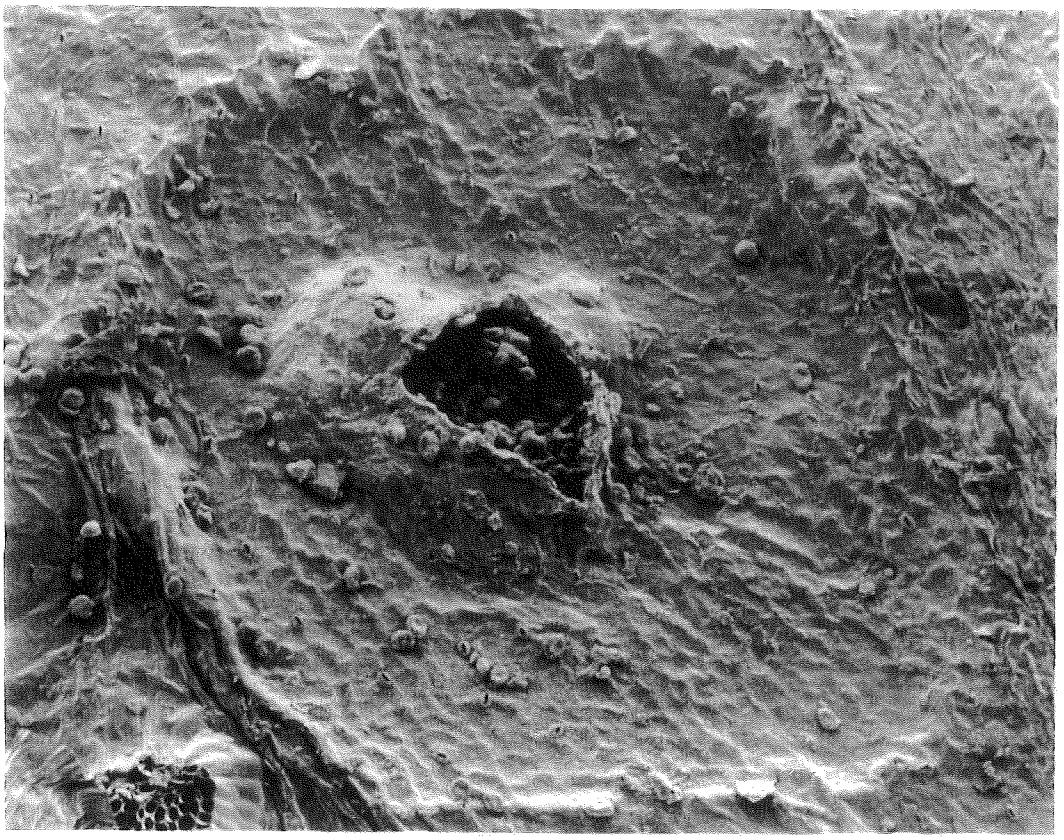
Fig 4.1 **Secondary pathogen**
SCANNING ELECTRON MICROGRAPH (x 100)
Necrotic lesion caused by a secondary pathogen invasion of the uredinium in the centre.

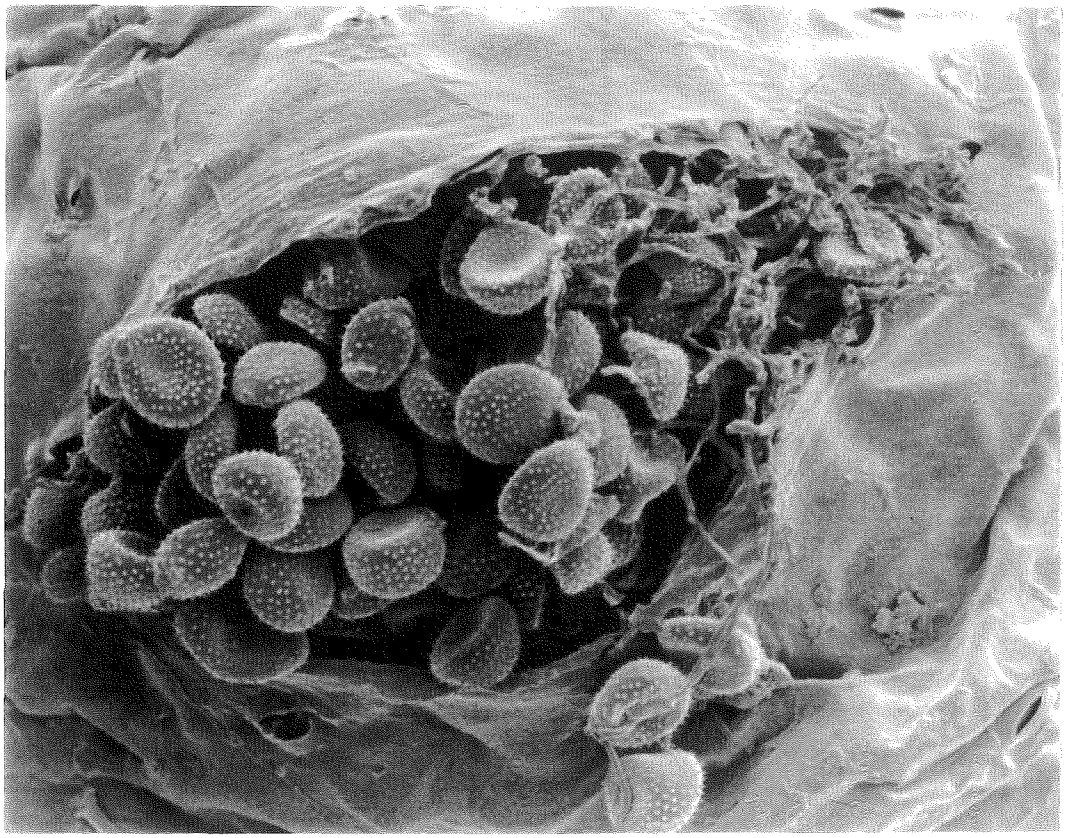
200μm

Fig 4.2 **Hyperparasite secondary pathogen**
SCANNING ELECTRON MICROGRAPH (x 500)
An unidentified fungus has produced hyphae within a rust uredinium and is apparently hyperparasitising the rust.



50μm





Generally more secondary pathogens were present in patches surrounded by moss. The presence of moss implies both the presence of high moisture at some times, and the ability of a site to remain damp, which would provide high humidity to aid growth of the secondary pathogens. Moist conditions, particularly when warm favour the growth of most facultative fungal pathogens and have been specifically noted as favouring secondary pathogens entering through rust sori (Hallett *et al.*, 1990). Field observations in other areas during autumn indicated that levels of secondary pathogens in the rust sori appeared to be higher in damper areas, particularly among moss. Secondary pathogens were also observed in drier areas, including the very exposed (to the sun) patches along the Innocent Railwayline in Holyrood Park.

IV.4 COVERED SORI

IV.4.1.a Introduction

During surveys throughout Europe, an amorphous covering on uredinia of rust on *H. pilosella* was occasionally observed. The covering appeared to be most common after a period of stress caused in particular by dryness or cold. Fluctuations in levels of covered sori were monitored from early summer through to the next spring at one site and factors affecting the presence of covered sori during summer were investigated. Glasshouse and laboratory work investigated the nature and reversibility of the phenomenon.

IV.4.1.b Methods

During the survey of 236 patches of *H. pilosella* in Holyrood Park (Section IV.2), the estimates of numbers of covered sori per leaf for each patch were recorded. As according to the methods in Section IV.2.1.b(i), stepwise multiple regression was applied to elucidate the environmental factors significantly affecting the presence of covered sori. The seasonal monitoring described in Section IV.1, also carried out at Holyrood Park, included the counting of covered sori on 50 rosettes in each of ten patches of *H. pilosella*. The results are presented in this section.

Leaves, cleared and fluorescently stained by the method of Helfer (1986), were examined under a Zeiss Axiophot light microscope. Specimens were prepared for standard and cold stage scanning electron microscopy with a Zeiss Digital Electron Microscope X2000.

IV.4.1.c Results

It was not clear whether the amorphous substance covering sori was of plant origin or from either the rust or other fungi. The process was reversible and hyphae beneath covered sori could survive (Fig 4.5).

Field observations indicated that covered sori were most common during mid-summer and again through the winter months. A light brown covering formed over the sorus often increasing to cover the entire sorus. Affected sori are shown in Figs 4.3 and 4.7. When plants were placed outside for a week in frosty conditions, a white substance protruded from the surface of uredinia (Fig 4.4). Fig 4.6 shows a rust sorus that was exposed to frosty conditions also for one week, urediniospore production was very limited and hyphal tips were exposed.

It was observed that with changes in environmental conditions such as rain after a dry spell or warmth after a cold period, the sori could recommence the production of spores and rupture the covering.

The average numbers of covered sori present on rosettes of ten patches over the course of a year are presented in Table 4.6. In absolute terms, covered sori were more common in the mid to late summer. Covered sori were however significant in relative terms over the winter when overall sori numbers were heavily reduced.

In the survey of 236 patches at Holyrood Park, an overall average of 1.6 covered sori per leaf were present on the patches surveyed. The maximum presence recorded was an average of ten covered sori per leaf. Covered sori were present on 94.5% of patches and only one patch which hosted actively sporulating sori lacked covered sori.

Table 4.6. Average numbers of covered sori present on rosettes of ten Holyrood Park patches over the course of nine months. (The percentage of covered sori out of total sori, including sporulating and secondary pathogen infected sori, is given in bold).

Patch		Date							
		Mid June 1993	Early July 1993	Late July 1993	Late Aug. 1993	Mid Sept. 1993	Mid Nov. 1993	Early Jan. 1994	Early Mar. 1994
Innocent Railwayline (i)	no. sori	3.6	0.2	0	17	2.2	0.1	0.2	2.6
	% of total	**4**	**0.2**	**0**	**36**	**74**	**0.8**	**84**	**17**
Innocent Railwayline (ii)	no. sori	5.0	3.5	0	28	5.5	1.1	0.5	2.0
	% of total	**14**	**8**	**0**	**83**	**27**	**42**	**100**	**10**
Samson's Ribs	no. sori	0	0	0	0.9	3.3	0	0.3	5.2
	% of total	**0**	**0**	**0**	**2**	**19**	**0**	**44**	**23**
The Hawse	no. sori	1.0	6.6	0	0.7	2.3	0	0.2	1.0
	% of total	**19**	**46**	**0**	**88**	**63**	**0**	**100**	**18**
South Quarry M	no. sori	4.1	20	0	6.9	6.5	0.1	0.1	14
	% of total	**46**	**76**	**0**	**60**	**56**	**6**	**18**	**91**
Cat Nick Slope	no. sori	0	0	0	1.5	3.9	0.1	1.2	1.2
	% of total	**0**	**0**	**0**	**4**	**15**	**2**	**100**	**46**
Galloping Glen N	no. sori	0.5	6.8	1.2	17	4.6	0.5	0	7.4
	% of total	**5**	**32**	**11**	**76**	**45**	**43**	**0.0**[a]	**71**
Galloping Glen S	no. sori	0.8	5.8	0	13	2.5	0	0	10
	% of total	**7**	**30**	**0**	**84**	12	**0.0**[a]	**0.0**[a]	**93**
Girnal Crag	no. sori	0	0	0	6.2	3.7	0	0.3	10
	% of total	**0**	**0**	**0**	**21**	**32**	**0**	**74**	**85**
Dunsapie Crag	no. sori	0.3	0	0	0.4	0.2	0	0.2	1.0
	% of total	**1**	**0**	**0**	**2**	**2**	**0**	**100**	**98**
Overall	no. sori	15	43	1.2	92	35	3	3	54
	% of total	**3**	**7**	**1**	**35**	**26**	**6**	**74**	**90**

[a] There were no active sori evident on the sampled rosettes during the marked sample times at these patches

Multiple regression of the data from the 236 Holyrood Park patches showed a significant relationship between the number of covered sori and the number of actively sporulating sori ($p<0.001$). The relationship with rust percent explained 15.1% of the variation, but higher presence of rust sori could just mean an increased number which are covered. Therefore the percentage rust infected plants in a patch was fitted to the model inclusion of site factors. With percentage infected plants included, the site location of the patch was significant ($p<0.001$) and the model accounted for 33.3% of the variation in covered sori levels.

With percent rust accounted for in the multiple regression model, the proportion of surrounding tall grass (inverse relationship, $p=0.014$) and level of soil organic matter (direct relationship, $p<0.001$) were significant. With percent rust and area included in the model, tall grass was still a similar significance ($p=0.016$) and soil organic matter (at $p=0.02$) lost some significance but still remained within the 95% confidence level.

IV.4.1.d Discussion

Presence of covered sori was interpreted as a response to dry or cold conditions and since sporulation could sometimes resume it appears to enhance survival of a sorus through adverse conditions. The phenomenon was very common in the Holyrood Park area and was also observed in other parts of Europe.

Perhaps the most important aspect of the field study results from the survey of 236 *H. pilosella* patches in Holyrood Park was the negative correlation with tall grass. Late in the summer, rust infections on *H. pilosella* rosettes surrounded by tall grass were observed to generally remain more active presumably due to the lower water stress either affecting plant condition or the rust directly. It is likely that in the New Zealand high country rust sori are more likely to remain active over the summer on rosettes close to tussock tillers and other vegetation. The relationship of high levels of covered sori related to high levels of soil organic matter was significant but the reason is unclear. Statistical significance of soil organic matter was reduced by including, in the model, area in which the patch was located, indicating that the correlation was partially indirect.

The composition of the covering was not ascertained and may have been of fungal or plant origin. Cross sections of affected sori show that host cells were not always in close proximity to the covering.

Hyperparasites or secondary pathogens may result in crust formation on rust fungi sori, for example *Verticillium lecanii* (A. Zimmerm.) Viégas produces a white crust on uredinia and *Tuberculina vinosa* Sacc. (=*T. maxima* Rostr.) produces violet or brownish crusts on pycnia, aecia and telia of microcyclic rusts (Kranz, 1981). The species are also able to infect other sori types (E. McKenzie pers. comm.). Generally there was no evidence of hyperparasite fungal mycellium in the covered sori examined under the SEM.

The potential advantage of covered sori for protection of the rust from adverse environmental conditions requires investigation; open sori are typically vulnerable to cold damage (Section I.4.7).

Fig 4.3 **Covered sori formation - early**
MACROSCOPE (x40)
Early stage of covered sori. Note the light brown amorphous covering on the large sorus on the left.

Fig 4.4 **Covered sori formation - formation**
MACROSCOPE (x 40)
Changes in rust infected *H. pilosella* plants placed outside the glasshouse in cold and dry exposed conditions. The first reaction noted was the production of a white substance, protruding from most uredinia. One month later these sori were similar in appearance to covered sori shown in Fig 4.3.

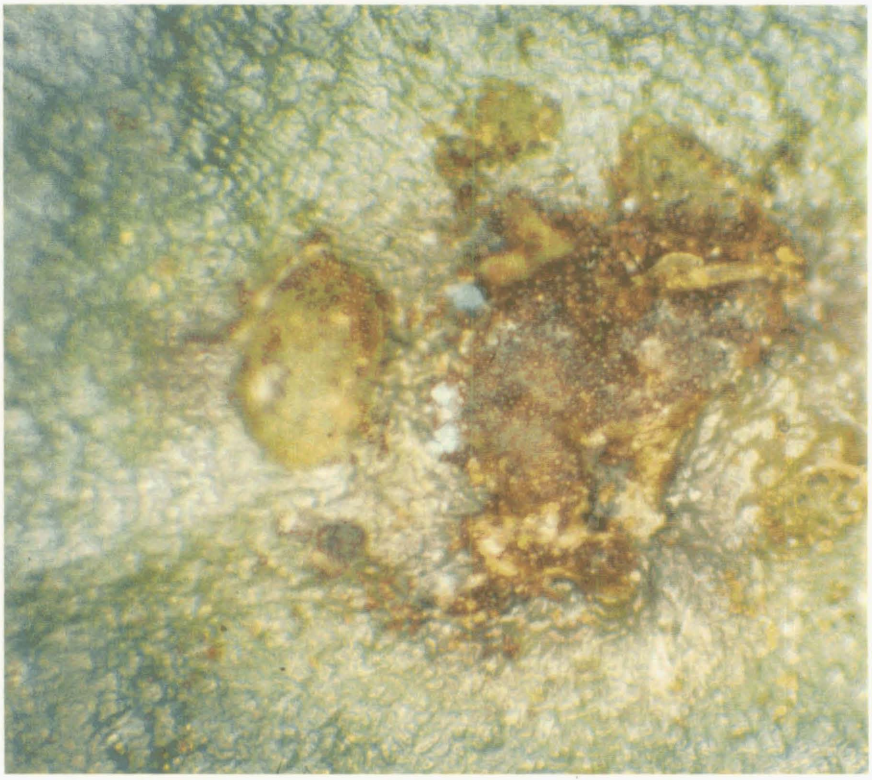


Fig 4.5 **Covered sori formation - encapsulated hyphae**
SCANNING ELECTRON MICROGRAPH (FREEZE FRACTURE) (x 500)
A freeze fracture near a covered uredinium shows apparently healthy rust hyphae within leaf tissue.

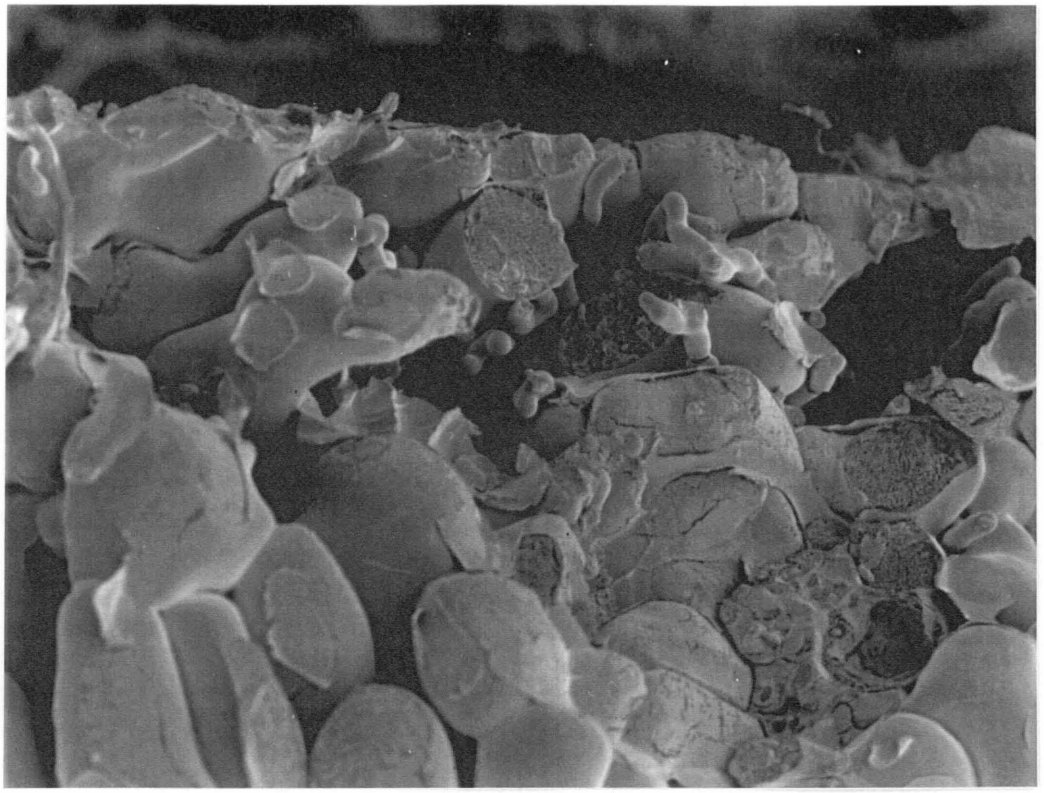
50μm

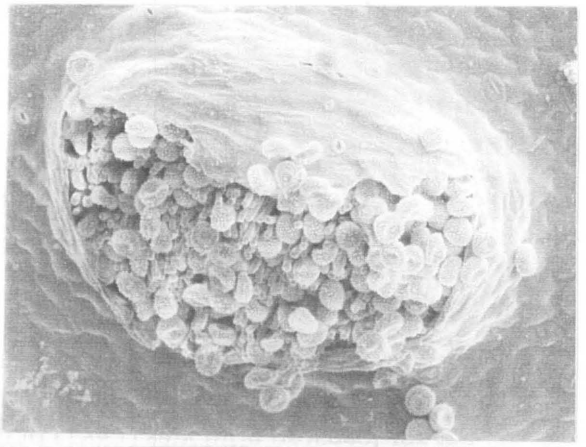
Fig 4.6 (middle) **Covered sori formation - reduced urediniospore production**
SCANNING ELECTRON MICROGRAPH (x 250)
Hyphal tips are visible in a uredinium which has been exposed to frosty conditions for one week. Uredinospore production was limited.

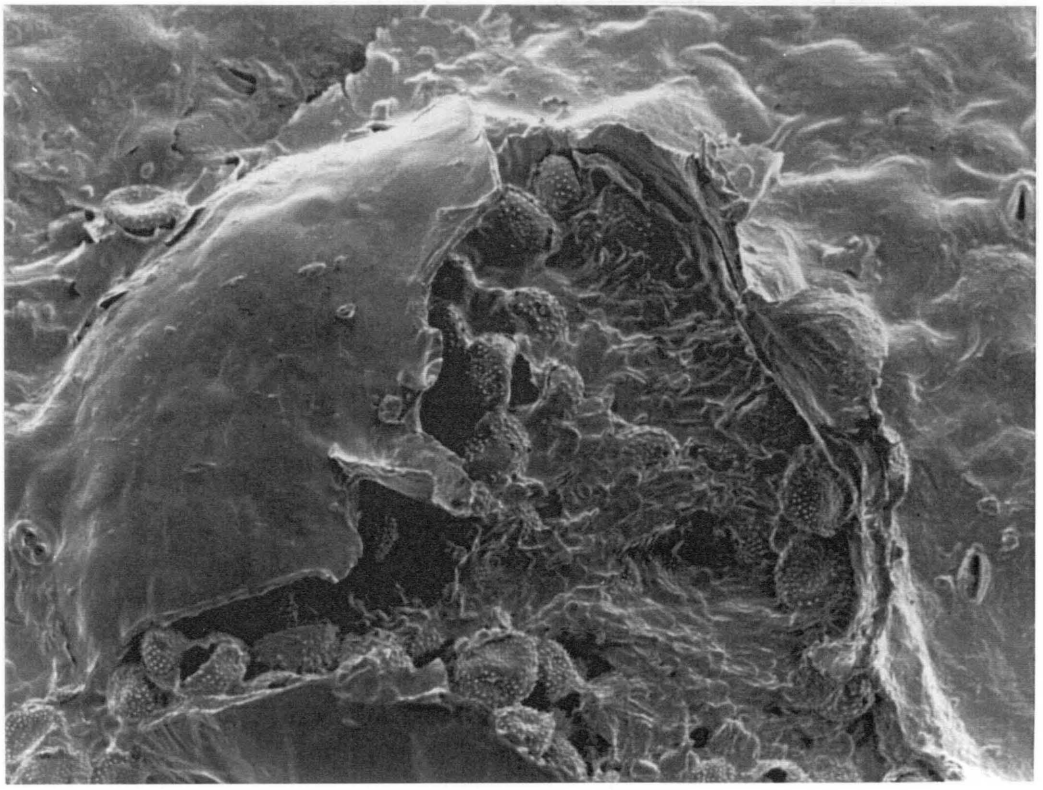
100μm

Fig 4.7 **Covered sori formation - covered uredinium**
SCANNING ELECTRON MICROGRAPH (x360)
An amorphous substance covers most of the surface of this uredinium.

50μm







CHAPTER V: COMPARATIVE PATHOGENICITY OF RUST ISOLATES

V.1 INTRODUCTION

A large series of experiments was carried out during the two year period in Europe to test the relative pathogenicity of rust isolates. Infectivity, based on the number of sori produced by each isolate, was the principal factor measured. Qualitative observations were made on size of sori, and the presence of any resistant reactions. The latent period, between penetration and sori production, was also noted for many isolates.

Many experiments were performed on detached leaves kept on water agar in petri dishes. Such experiments allowed a more efficient use of inoculum and resulted in a higher percentage of successful infections than the inoculation of whole plants.

V.1.1 PROTOCOL OF SCREENING OF RUST ISOLATES

Field collections of isolates were made in several geographical areas. The variation in infectivity within the major collections, from Britain, Ireland and European continental countries was examined. Subsequently comparisons between isolates from each of these three collections were compared. Screening was on detached leaves or whole rosettes, with a range of New Zealand *H. pilosella* collections.

The outline of screening was as follows.

1 1992: Comparison of 70 British isolates on detached leaves.
2 1992: Comparison of seven British isolates on whole plants.
3 1993: Comparison of 1073 British isolates on detached leaves and whole plants.
4 1993: Comparison of 180 Irish isolates on detached leaves and whole plants.
5 1993: Comparison of 100 continental European isolates on whole plants.
6 1993: Comparison between selected isolates from the various collections.

Due to the number of isolates collected in 1993, screening involved few replicates or host types for each isolate. The main objective was to select those isolates most pathogenic on New Zealand *H. pilosella.*

V.1.2 COMPARISON OF 70 BRITISH ISOLATES ON DETACHED LEAVES

Seventy isolates from throughout Britain were initially screened on the basis of the number of sori produced on detached leaves of pentaploid New Zealand *H. pilosella* ex HpilHAs. Further isolates were collected during 1992 when this experiment was conducted but they were screened in subsequent experiments (see Section V.1.4).

V.1.3 COMPARISON OF SEVEN BRITISH ISOLATES ON WHOLE ROSETTES EX HpilHOs

Seven rust isolates were tested on whole *H. pilosella* rosettes ex locality HpilHOs for further elucidation of the infectivity ranking. The locality HpilHOs was selected because it appeared to be the most susceptible locality of *H. pilosella* of the five briefly tested in Section III.5.1. A comparison with the rankings obtained using detached leaves was possible as all but one of the isolates were included in the previous detached leaf experiment (Section V.1.2). An isolate of unknown origin (isolate SELF1) that had spread onto a rosette of HpilLRu locality was also included.

V.1.4 SCREENING OF BRITISH ISOLATES ON DETACHED LEAVES

In the latter part of 1992 to 1993, a further 1073 rust isolates were collected from Britain, particularly Scotland. All of the isolates were screened for infectivity on detached leaves of two New Zealand *H. pilosella* collections, HpilHAs (pentaploid cytotype) and HpilGPs.

V.1.5 SCREENING OF BRITISH ISOLATES ON WHOLE ROSETTES

Subsequent to previous screening, all isolates producing sori on the detached leaves and the top ranking isolates from 1992 British collections being inoculated onto rosettes of *H. pilosella* ex HpilHOs. The results were used to rank British isolates for infectivity on New Zealand *H. pilosella*.

V.1.6 INITIAL SCREENING OF IRISH ISOLATES

From mid March to mid April 1993, 180 isolates of rust were collected from the Republic of Ireland. The isolates were screened for infectivity on whole rosettes of New Zealand *H. pilosella* HpilHOs.

V.1.7 SCREENING OF 32 IRISH ISOLATES ON DETACHED LEAVES

Irish isolates that produced sori in previous screening were tested on detached leaves of two collections, HpilHAs (pentaploid cytotype) and HpilLCo. The proportion of leaves infected and the number of sori produced were recorded.

V.1.8 COMPARISON OF FIVE IRISH ISOLATES ON WHOLE ROSETTES AND DETACHED LEAVES

The five Irish isolates with the highest rank from previous detached leaf screening were inoculated onto a range of rosettes and detached leaves from five New Zealand localities. The isolates were compared with two British isolates, BRIT12 and BRIT32.

V.1.9 COMPARISON OF CONTINENTAL EUROPEAN ISOLATES

A collection of 100 isolates from various regions of Europe were tested on New Zealand *H. pilosella* rosettes. The regions included Denmark, northern Germany, southern Germany, Sweden, Switzerland, and the Alps of Italy and France.

V.1.10 COMPARISONS BETWEEN RUST COLLECTIONS

An experiment was designed to rank the most infective isolates from each of the previous major collections. The highest ranked British isolate and the highest ranked Irish isolate were compared against the European isolates that had produced infection on New Zealand rosettes.

V.2 METHODS

V.2.1 COMPARISON OF 70 BRITISH ISOLATES ON DETACHED LEAVES

The experimental design was a factorial design of ten randomly selected isolates of a range of 70 on each day by ten replicates of detached leaves per day by four days per week over 12 weeks (48 experimental days). During spring and early summer of 1992, the 70 isolates were collected from throughout Britain. Inoculum for the screening was taken from original potted plants. Infectivity of each isolate was compared on detached leaves of pentaploid New Zealand *H. pilosella* ex HpilHAs. An average of 700 detached leaves for each isolate were inoculated over 48 days. The assumption of the rosettes being pentaploid was based on chromosome counting (with five countable mitotic spreads from each of five root tips) of one seedling per capitulum of seed used to produce the seedlings. Leaves were taken from a selection of fifty rosettes.

Leaves of similar surface area were selected and trimmed to equal sizes. Leaves were the 4th to the 10th youngest open leaves from a rosette and showed no sign of senescence. Ten leaves were evenly spaced in each petri dish. Ten petri dishes were prepared for each day and pre-incubated in the dark for two days (ca. 10°C to 20°C) following the findings of Scott (pers. comm. 1995a).

Ten isolates were selected at random for each day of an experiment from the range of isolates with sufficient inoculum. It was possible for an isolate to be selected more than once in a day. Inoculation was carried out using moist cotton buds as described in Section II.2.4.a.

Each of the leaves in a petri dish was inoculated with a different rust isolate. After inoculation, petri dishes were placed in the glasshouse within cubicles of translucent polypropylene plastic sheeting, 30 cm high, uncovered. The cubicles provided diffuse light, reducing light gradients that might occur in the glasshouse. Petri dishes were placed in randomly chosen orientations.

Leaves were observed for the production of sori over a period of four weeks post inoculation. The relative infectivity of isolates was measured according to a rank for each isolate, based on number of sori produced, for each experimental day in which the isolate was tested. The mean rank for an isolate was derived from the sum of the ranks (less 1) in each day divided by the number of days in which the isolate was tested.

$$\text{Mean Rank} = \sum (r_x - 1)\, n^{-1}\, d^{-1}$$

where r_x= the rank for an isolate in day x, based on the number of sori produced.

n = the number of isolates tested per day.

d = the number of days for which the isolate was tested.

In the case of an isolate producing no infection during an experiment, r_x a value of 11. The value for n was usually 10. When an isolate was selected more than once in a day, the rank for the highest result was calculated with n=10; the second highest result was calculated without the former result , thus n=9; and if a third result was present, it would be ranked without the two greater results, thus n=8.

Multiple regression was employed to test the significance of differences between isolates and between treatment days in the numbers of sori produced per leaf and the number of leaves infected. ANOVA was performed on the results of each experimental day to test for differences between the isolates in the number of sori produced per leaf.

V.2.2 COMPARISON OF SEVEN BRITISH ISOLATES ON WHOLE ROSETTES EX HpilHOs

Experimental design was seven rust isolates by five occasions on successively older host plants (5, 6, 9, 10 and 11 weeks old) with five rosette replications at each stage. On each occasion five replicates of *H. pilosella* rosettes ex HpilHOs were inoculated with each isolate and five rosettes were used as controls. Inoculation was made using

moist cotton buds according to the methods in Section II.2.4.b. The number of sori produced was counted on each rosette after four weeks. The isolates were BRIT2, BRIT12, BRIT32, BRIT52 and SELF1.

V.2.3 SCREENING OF BRITISH ISOLATES ON DETACHED LEAVES

Experimental design was 1073 rust isolates tested on a single detached leaf of each of two *H. pilosella* host collections (HpilHAs pentaploid, and HpilGPs). The HpilHAs rosettes used to provide the leaves were all rosettes that had been grown from seed of the same capitulum as seed that had produced verified pentaploid plants. Detached leaves were the 4th to 10th youngest of rosettes greater than eight weeks old. Inoculation was made with cotton buds as described in Section II.2.4.b. The screening procedure took place over several months from early June till September, 1993.

If an isolate failed to infect either of the detached leaves tested, the isolate was not considered in further experimentation. This severe screening method was considered necessary due to technical constraints of handling and maintaining a large number of isolates and an assumption that a highly virulent isolate would give rise to infection on the first attempt at inoculation.

V.2.4 SCREENING OF BRITISH ISOLATES ON WHOLE ROSETTES

The 515 British isolates that gave infection in the detached leaf screening, together with the top ten isolates according to the 1992 detached leaf series, were compared. The experimental design was 525 isolates by two *H. pilosella* rosettes of each of two localities (HpilHOs and HpilLRd). Inoculations were made using moist cotton buds according to the methods of Section II.2.4.b. Rosettes were monitored for the production of sori.

V.2.5 INITIAL SCREENING OF IRISH ISOLATES

The 180 isolates collected from Ireland were inoculated onto one rosette each of 3.5 month old *H. pilosella* ex HpilHOs, in June 1993. Inoculations were made with moist cotton buds according to the methods in Section II.2.4. Urediniospores from only ten average sized (ca. 0.5 mm diameter) sori were used for each isolate, as some isolates were not represented by many sori after transport and quarantine. Rosettes were monitored for the production of sori. All isolates giving infectivity were selected for further screening (Section V.2.6).

V.2.6 SCREENING OF 32 IRISH ISOLATES ON DETACHED LEAVES

Experimental design was 32 Irish rust isolates plus two British isolates (BRIT12 and BRIT52) tested on eight leaf sections of each of two *H. pilosella* localities (HpilMAs and HpilLCo) in July 1993. Sixteen water agar (0.95%) plates were prepared. Thirty two 2 cm^2 leaf sections were placed in each plate; the leaf sections were each from separate individuals. Eight plates were filled with leaf sections of *H. pilosella* ex HpilMAs and the remaining eight plates had *H. pilosella* ex HpilLCo leaf sections. The host rosettes were 5.5 months old. Each isolate was inoculated onto one randomly selected leaf section in each plate, using moist cotton buds according to the method in Section II.2.4.b. Leaf sections were monitored for the production of sori. The isolates giving the highest rates of infection were selected for the next stage of Irish isolate screening (Section V.2.7).

V.2.7 COMPARISON OF FIVE IRISH ISOLATES ON WHOLE ROSETTES AND DETACHED LEAVES

The selected Irish isolates and two British isolates, BRIT12 (highest ranked from Britain) and BRIT32, were tested against two whole rosettes and four detached leaves of five localities of New Zealand *H. pilosella* in September 1993. These localities were HpilLRd, HpilGPs, HpilMJt, HpilHOs and HpilLRu. Rosettes of the HpilMJt and HpilLRu localities were grown from seed borne of the same capitula as seed used to produce rosettes for isozyme electrophoresis studies in St Andrews (see Section VII.2.6.c); the HpilMJt rosettes were siblings of the sample that matched most other localities in IDH banding; the HpilLRu rosettes were siblings of the sample that

displayed differences in IDH banding. Rosettes of these two localities and those of HpilLRd had been sown at the same time (six months prior to inoculation) and grown randomly intermingled. Rosettes of HpilGPs had been planted at the same time and grown in the same glasshouse area but not intermingled with other localities. The HpilHOs locality rosettes were eleven months old and had been used in a rust spread experiment (Section III.4.2) but had not been infected.

Eight 2 cm^2 leaf sections from rosettes of each locality were placed in each of four water agar (0.95%) plates, with two leaf sections per plate being controls. Each rust isolate was inoculated onto a separate leaf section of each locality in each agar plate. Inoculations were made using moist cotton buds according to the methods in Section II.2.4.a. Whole rosettes were inoculated according to the methods in Section II.2.4.b with two rosettes per locality treated as controls. Control leaves and rosettes were brushed with moist cotton buds lacking urediniospores. After inoculation, the rosettes were placed in randomly intermingled positions. Leaf sections and rosettes were monitored for the production of sori.

V.2.8 COMPARISON OF CONTINENTAL EUROPEAN ISOLATES

Experimental design was 100 rust isolates tested on two rosettes of *H. pilosella* ex HpilHOs. Urediniospores of isolates from Sweden and two of the French Alps isolates were collected fresh from transplanted original host plants. Isolates from Switzerland and Italy and the majority from France were represented in this screening by urediniospores from cotton bud collections within a month of collection (see Section II.2.2). Isolates ÉIRE45 (from Ireland) and BRIT12 (from Scotland) were inoculated onto two rosettes each, as comparisons. Urediniospores of these two isolates were taken from plants transplanted from the field and grown in the glasshouse for approximately two months.

Rosettes were eight weeks old, with at least six leaves. Fifty control rosettes treated with moist cotton bud lacking urediniospores were included to test for the presence of contamination from rust urediniospores of other isolates in the glasshouse. Otherwise,

inoculation methods were as outlined in Section II.2.4.b. The presence of sori was recorded after four weeks.

V.2.9 COMPARISONS BETWEEN COLLECTIONS

Two rosettes each of Twizel, Holbrook and Godley Peaks localities were inoculated with similar quantities of urediniospores of each of 100 European rust isolates grown on *H. pilosella* hosts ex HpilHOs. Urediniospores of isolates ÉIRE14 and BRIT12 were collected from hosts ex HpilHOs and each inoculated onto four rosettes, of each locality. Inoculation method was with moist cotton buds as described in Section II.2.4.b. Four rosettes of each locality were treated as controls with moist cotton buds lacking urediniospores.

The number of sori produced on each rosette after four weeks was counted. Ranking of isolates was based on the number of sori produced by the isolates on rosettes of three New Zealand *H. pilosella* localities.

V.3 RESULTS

V.3.1 COMPARISON OF 70 BRITISH ISOLATES ON DETACHED LEAVES

The ranking of each of the 70 isolates based on infectivity is presented in Table 5.1. All of the seventy isolates caused infection on the detached leaves though often on a low percentage of treated leaves. A lower mean rank indicated a larger number of sori produced.

Logistic regression was applied to the data to test the significance of observed differences in the proportion of leaves infected and the number of sori produced by isolates (Tables A.1 and A.2, respectively, in Appendix 1). Differences between isolates were highly significant for the proportion of leaves infected ($\log_{10}(x + 1^{-10})$ transformed) ($p=0.0001$) but lacked significance in terms of the number of sori produced ($p=0.601$).

Table 5.1. Ranking of the 70 British rust isolates tested on detached leaves, 1992. (Score is the result of calculations described in Section V.2.1).

RANK	CODE	SCORE	AVERAGE NO. SORI PRODUCED	%TREATED LEAVES INFECTED	NO. OF REPS
1=	BRIT 53	0.1278	114	70	8
1=	BRIT 12	0.1278	113	66	9
3	BRIT 21	0.2143	99	67	7
4	BRIT 26	0.2308	89	56	13
5	BRIT 32	0.2375	84	53	12
6	BRIT 57	0.2514	86	55	8
7	BRIT 60	0.2750	83	63	4
8	BRIT 52	0.2800	63	42	5
9	BRIT 62	0.3000	114	65	2
10	BRIT 64	0.3167	88	53	6
11	BRIT 7	0.3200	75	50	5
12=	BRIT 6	0.3500	83	51	6
12=	BRIT 51	0.3500	83	48	13
14	BRIT 28	0.3600	82	48	5
15=	BRIT 30	0.3750	83	63	4
15=	BRIT 61	0.3750	83	42	6
17	BRIT 3	0.3800	74	56	5
18	BRIT 2	0.3905	74	47	7
19=	BRIT 58	0.4000	87	58	4
19=	BRIT 36	0.4000	81	53	6
19=	BRIT 50	0.4000	70	50	6
22	BRIT 4	0.4027	75	49	14
23	BRIT 49	0.4083	29	25	4
24	BRIT 59	0.4111	62	40	6
25	BRIT 35	0.4143	59	41	7
26=	BRIT 39	0.4333	60	43	3
26=	BRIT 41	0.4333	55	33	6
28	BRIT 13	0.4425	58	41	7
29	BRIT 14	0.4429	74	53	7
30	BRIT 25	0.4444	60	46	12
31	BRIT 15	0.4500	80	50	6
32	BRIT 69	0.4563	72	49	9
33	BRIT 37	0.4642	71	46	9
34	BRIT 9	0.4700	57	40	11
35	BRIT 40	0.4728	62	40	9
36	BRIT 55	0.4778	42	32	6
37	BRIT 1	0.4857	62	43	7
38	BRIT 33	0.4954	64	33	12
39	BRIT 22	0.4957	57	37	13
40	BRIT 47	0.5000	52	34	5
41	BRIT 29	0.5100	48	34	5
42	BRIT 66	0.5200	47	31	10
43	BRIT 5	0.5268	39	29	7
44	BRIT 24	0.5300	54	40	5
45	BRIT 68	0.5333	43	40	4
46	BRIT 44	0.5357	50	39	7
47=	BRIT 20	0.5400	62	38	6
47=	BRIT 10	0.5400	45	32	5
49	BRIT 27	0.5491	54	40	12
50	BRIT 63	0.5625	54	44	8
51=	BRIT 23	0.5750	63	40	4
51=	BRIT 45	0.5750	45	31	14
53	BRIT 18	0.5800	49	36	5
54	BRIT 65	0.5857	31	28	8
55	BRIT 31	0.5929	61	37	7
56	BRIT 34	0.5938	49	35	8
57=	BRIT 17	0.5944	38	25	4
57=	BRIT 42	0.5944	37	28	6
59=	BRIT 56	0.6000	55	38	4
59=	BRIT 43	0.6000	49	38	4
59=	BRIT 11	0.6000	48	37	9
59=	BRIT 8	0.6000	43	30	6
59=	BRIT 46	0.6000	38	40	4
64	BRIT 70	0.6375	25	23	4
65	BRIT 16	0.6400	41	28	5
66	BRIT 67	0.6500	37	28	4
67	BRIT 48	0.6750	24	20	4
68=	BRIT 19	0.7333	48	30	7
68=	BRIT 54	0.7333	28	20	3
70	BRIT 38	0.7714	24	16	7

Average standard deviation for number of sori produced was 44 and for percent of treated leaves infected was 24.

The differences between experimental days and the interaction between experimental day and isolate were not statistically significant for either the proportion of leaves infected or the number of sori produced by the isolates.

Multiple regression demonstrated significant differences were found between rust isolates in terms of the percentage of leaves infected (log transformed, p=0.016) and the number of sori produced (p=0.028). No significant difference between weeks was demonstrated in the multiple regression analysis of percentage leaves infected (log transformed, p=0.073). The difference between weeks in the number of sori produced was only significant (p=0.003) when data were log transformed.

V.3.2 COMPARISON OF SEVEN BRITISH ISOLATES ON WHOLE ROSETTES EX HpilHOs

An average of 70% of rosettes were infected after inoculation. The percentages of plants infected and the average numbers of sori produced by each isolate are presented in Table 5.2. No control rosettes were infected with rust. Analysis of variance found a significant difference in the number of sori produced in each of the five experiments (p=0.0001) with a trend to increased sori number in the later experiments. The differences between isolates in sori produced were not statistically significant at the 95% confidence interval (p=0.06).

Table 5.2. Number of sori produced by rust isolates during infectivity tests on *H. pilosella* rosettes ex HpilHOs.

ISOLATE	% PLANTS INFECTED	AVERAGE NO. SORI	RANKING from Section (V.3.1)
BRIT52	84	138	7
BRIT12	80	113	1=
BRIT53	76	164	1=
BRIT32	76	58	5
SELF1	64	116	not ranked
BRIT55	60	177	35=
BRIT2	48	79	16

V.3.3 SCREENING OF BRITISH ISOLATES ON DETACHED LEAVES

Sori were produced by 515 (63%) isolates inoculated on to *H. pilosella* HpilHAs (pentaploid) detached leaves. Only two isolates produced sori on detached leaves of HpilGPs (Godley Peaks) rosettes. These isolates were BRIT60 (Pitlochry, UK) and BRIT138 (Salisbury Crags, Edinburgh, UK).

V.3.4 SCREENING OF BRITISH ISOLATES ON WHOLE ROSETTES

The results for ten most infective isolates from Section V.3.3 are presented in Table 5.3 with a ranking derived from the number of sori produced (full data in Appendix 2, Table A.3). Sori were produced on 77 HpilHOs and 86 HpilHOs rosettes, by a total of 135 (26%) of the isolates. The results of the ten top ranked isolates from the 1992 detached leaf screening (Section V.3.1) are presented in Table 5.3 below. Two of these isolates, BRIT12 and BRIT21, were the highest ranked in the present screening. The remaining eight isolates from the 1992 screening were widely distributed amongst the ranks though all of them produced infection on at least one rosette.

Table 5.3. Infectivity of rosettes by the ten most infective rust isolates in 1992 detached leaf tests.

Isolate Code	Ranking	Rank in Section V.3.1	No. of Sori on HpilLRd	No. of Sori on HpilHOs	Average No. of Sori
BRIT12	1	1=	58	38	48
BRIT21	2	3	47	48	47.5
BRIT64	42=	10	0	50	25
BRIT53	45=	1=	49	0	24.5
BRIT26	58=	4	42	0	21
BRIT60	60	7	0	41	20.5
BRIT62	70=	9	34	0	17
BRIT34	90	5	0	26	13
BRIT57	126=	6	7	0	3.5
BRIT52	130=	8	0	3	1.5

V.3.5 INITIAL SCREENING OF IRISH ISOLATES

Thirty-two isolates infected the HpilHOs rosettes and produced sori. The latent period of these isolates was (10 minimum-) 13 mean (-22 maximum) days. The 32 isolates were all tested in Stage 2.

V.3.6 SCREENING OF 32 IRISH ISOLATES ON DETACHED LEAVES

All isolates produced sori on the detached leaves of both localities. There was significant variation in the numbers of sori produced by each isolate ($P<0.001$) but no statistically significant differences between the numbers of sori produced on the two host localities or on the eight replicate plates. The results are presented in Table 5.4.

Table 5.4. Infection of detached leaves of two *H. pilosella* localities with 32 Irish and two British rust isolates.

Isolate	Infection of HpilMAs		Infection of HpilLCo	
	no. lvs infected (out of 8)	average no. sori	no. lvs infected (out of 8)	average no. sori
BRIT52	2	0.7	5	3.1
BRIT12	5	3.4	7	9.4
ÉIRE14	8	23.6	8	20.1
ÉIRE45	8	21.5	8	17.3
ÉIRE32	8	9.0	8	16.0
ÉIRE66	8	14.8	7	14.6
ÉIRE29	7	14.6	7	11.6
ÉIRE79	7	8.5	7	10.0
ÉIRE39	7	9.9	6	7.5
ÉIRE13	7	6.9	6	8.0
ÉIRE48	6	12.1	7	8.0
ÉIRE12	6	11.5	7	10.3
ÉIRE41	6	9.6	7	9.9
ÉIRE19	6	9.5	7	14.4
ÉIRE90	6	9.1	7	11.1
ÉIRE15	6	2.9	7	10.6
ÉIRE88	6	19.0	6	7.4
ÉIRE107	6	11.0	6	10.8
ÉIRE9	6	10.6	6	14.1
ÉIRE17	6	10.6	6	9.6
ÉIRE108	6	8.9	6	9.3
ÉIRE75	6	14.4	5	9.5
ÉIRE56	5	12.4	6	10.6
ÉIRE74	5	6.0	6	7.9
ÉIRE78	5	5.3	6	7.5
ÉIRE44	4	8.8	7	9.5
ÉIRE4	4	2.3	7	8.8
ÉIRE72	4	5.6	6	6.8
ÉIRE68	4	4.5	6	8.0
ÉIRE11	4	2.6	6	3.3
ÉIRE10	4	1.5	6	5.0
ÉIRE105	4	6.0	5	5.1
ÉIRE136	3	1.9	5	6.3
ÉIRE36	3	2.0	3	2.3

Average CV (standard deviation / mean) of values were 102 and 114 for localities HpilLCo and HpilHAs respectively.

V.3.7 COMPARISON OF FIVE IRISH ISOLATES ON WHOLE ROSETTES AND DETACHED LEAVES

All of the isolates produced sori on almost all of the host localities, the sole exception being that no isolate successfully infected hosts from locality HpilGPs. Results for detached leaves and for rosettes are presented in Table 5.5 and Table 5.6 respectively (with full data in Appendix 3, Table A.4). The uninoculated control rosettes and leaf sections did not develop sori.

Table 5.5. Average number of sori (absolute values) produced on detached leaves of New Zealand *H. pilosella* inoculated with Irish and British rust isolates.

ISOLATE		HOST SOURCE HpilLCo	HpilGPs	HpilMJt[a]	HpilHOs[a]	HpilLRu[a]	Mean Rank[b]
ÉIRE14	no. of sori	37	0	54	79	40	1 a
	leaves infected	4	0	4	4	4	
ÉIRE 29	no. of sori	22	0	44	24	30	2 ab
	leaves infected	4	0	4	4	4	
ÉIRE 32	no. of sori	15	0	14	36	20	3 ab
	leaves infected	4	0	4	4	4	
ÉIRE 45	no. of sori	17	0	20	28	37	4 ab
	leaves infected	4	0	4	4	4	
ÉIRE 66	no. of sori	12	0	9	22	24	5 bc
	leaves infected	3	0	4	4	3	
BRIT12	no. of sori	16	0	19	17	16	6 cd
	leaves infected	3	0	3	3	3	
BRIT32	no. of sori	11	0	25	9	10	7 d
	leaves infected	2	0	2	3	2	
Signif.[c]	no. of sori	b	c	a	a	a	

[a] Rosettes from these localities correspond to variants identified by isozyme electrophoresis; see text for details.
[b] The letters are significance indicators for the mean numbers of sori produced by each isolate. Isolates sharing a similar letter are not statistically significantly different.
[c] These are significance indicators for the mean numbers of sori produced on each host population. Populations (host localities) sharing a similar letter are not statistically significantly different.

Analysis of variance demonstrated that the differences between source of inoculated rosette as well as the source of inoculum were statistically significant. Significant differences (95% confidence level) are indicated in Table 5.6. When the results for locality HpilGPs were not included, the differences between other localities were no longer statistically significant.

Table 5.6. Average number of sori (absolute values) produced on rosettes of New Zealand *H. pilosella* inoculated with Irish and British rust isolates.

ISOLATE		HOST SOURCE HpilLCo	HpilGPs	HpilMJt[a]	HpilHOs[a]	HpilLRu[a]	Mean Rank
ÉIRE14	no. of sori	140	0	58	63	59	1 a
	rosettes infected	2	0	1	2	2	
ÉIRE 29	no. of sori	120	0	37	36	64	2 a
	rosettes infected	2	0	1	2	2	
ÉIRE 32	no. of sori	120	0	75	34	50	3 a
	rosettes infected	2	0	1	2	2	
ÉIRE 45	no. of sori	42	0	82	66	20	4 ab
	rosettes infected	2	0	2	2	1	
ÉIRE 66	no. of sori	67	0	42	33	40	5 ab
	rosettes infected	2	0	1	1	2	
BRIT12	no. of sori	24	0	7.5	29	20	6 b
	rosettes infected	1	0	1	2	2	
BRIT32	no. of sori	20	0	4	31	12	7 b
	rosettes infected	2	0	1	1	1	
Signif.[c]	no. of sori	a	c	a	a	b	

[a] Rosettes from these localities correspond to variants identified by isozyme electrophoresis; see text for details.

[b] The letters are significance indicators for the mean numbers of sori produced by each isolate. Isolates sharing a similar letter are not statistically significantly different.

[c] These are significance indicators for the mean numbers of sori produced on each host population. Populations (host localities) sharing a similar letter are not statistically significantly different.

V.3.8 COMPARISON OF CONTINENTAL EUROPEAN ISOLATES

Twenty-five of the isolates infected inoculated rosettes, isolate codes are given for each of these isolates in Table 5.7 with an indication of the percentage of infective isolates from each geographical region. The only isolates that infected on both of the inoculated rosettes were the British Isles isolates, ÉIRE14 and BRIT12. No infection was noted from the two French Alp isolates from transplanted rosettes. Overall, 25% of the tested isolates gave some infection. No signs of resistance were noted on any of the inoculated rosettes.

Table 5.7. Continental European rust isolates displaying infectivity on Holbrook rosettes.

Region	No. Isolates Collected	Sufficient Inoculum	Isolates Infecting[b]	% Isolates Infecting
Sweden	120	11	322;341;394;721;805	45
N. Germany	4	4	154;182	50
S. Germany	9	9	15	11
Switzerland[a]	134	46	17;21;52;120;129;172;183;188	17
France[a]	54	25	4;58;159;160;162;262;270;417	32
Italy[a]	12	2	-	0
Denmark	3	3	21	33

[a] Most isolates screened from these regions were from cotton bud collections.
[b] Isolates are indicated by their code number for each region.

V.3.9 COMPARISONS BETWEEN COLLECTIONS

Most isolates were capable of infecting some of the inoculated rosettes. No isolate infected all rosettes that were inoculated. Only one isolate, ÉIRE14, infected a rosette from HpilGPs. The average numbers of sori produced by the isolates on rosettes of the three localities are presented in Table 5.8. An analysis of variance on the number of sori (square root transformed) produced on HpilTWz and HpilHOs rosettes showed significantly more sori were produced on HpilTWz than on HpilHOs rosettes ($p=0.003$) and statistically significant differences were also seen between the numbers of sori each isolate produced ($p=0.0001$). ÉIRE14 produced significantly more sori on HpilTWz and HpilHOs rosettes than any other isolate ($p<0.05$). Isolate BRIT12 was ranked second but was not significantly different from isolate SVER341 in terms of sori produced. The statistical differences between isolates is shown in Table 5.8, based on least significant difference.

Table 5.8. Inoculations of European rust isolates onto *H. pilosella* from three New Zealand localities. (Average number of sori).

ISOLATE	Host Locality				
	HpilTWz	HpilHOs	HpilGPs	Average	Ranking[a]
ÉIRE14	47.8	32.8	1.5	40.3	a
BRIT12	20.0	12.5	0	16.3	b
SVER341	9.5	6.5	0	8.0	bc
FRAN4	12.0	2.0	0	7.0	cd
FRAN262	16.5	0	0	8.3	cde
SUIS183	9.5	0	0	4.8	cdef
SUIS17	0	6.0	0	3.0	def
FRAN162	6.0	0	0	3.0	def
SVER721	5.5	0	0	2.8	def
SVER805	4.0	0	0	2.0	def
SUIS52	0	4.0	0	2.0	def
DEUTs15	3.0	0	0	1.5	def
SUIS120	3.0	0	0	1.5	ef
SUIS129	0	3.0	0	1.5	ef
SVER322	0	1.0	0	0.5	f
DEUTn154	0.5	0	0	0.3	f
DANM21	0	0	0	0	f
FRAN159	0	0	0	0	f
FRAN160	0	0	0	0	f
FRAN270	0	0	0	0	f
FRAN417	0	0	0	0	f
FRAN58	0	0	0	0	f
DEUTn182	0	0	0	0	f
SUIS172	0	0	0	0	f
SUIS188	0	0	0	0	f
SUIS21	0	0	0	0	f
SVER394	0	0	0	0	f

[a] Ranking, derived from least significant differences of square root transformed data, is according to the average number of sori produced on HpilTWz and HpilHOs rosettes. Isolates with the same letter are not significantly different (95% confidence interval).

V.4 DISCUSSION

V.4.1 COMPARISON OF 70 BRITISH ISOLATES ON DETACHED LEAVES

Most of the top ranked isolates were from Scotland including all of the top 18. Only 15 isolates (21%) were, however, collected from England and Wales and therefore it might be expected that there would be a higher chance of collecting more infective isolates from Scotland. The differences in infectivity between isolates in terms of the proportion of leaves infected were insufficient to discriminate between closely ranked isolates.

Although the detached leaves were all from pentaploid rosettes, genetic uniformity was doubtful; isozyme electrophoresis indicated that there was genetic variation within pentaploid *H. pilosella* from locality HpilHAs (Section VII.2.6.c).

V.4.2 COMPARISON OF SEVEN BRITISH ISOLATES ON WHOLE ROSETTES EX HpilHOs

The three top ranked isolates, in terms of the number of rosettes infected, included the two top ranked isolates from Section V.3.1., BRIT12 and BRIT53. The significant linear contrast between the two groups of isolates based on their ranking in the detached leaf screening indicated some degree of correlation between the ranking of infectivity on detached leaves ex HpilHAs and that on whole rosettes ex HpilHOs. Although glasshouse conditions may have changed between experiments, plant age may have been a factor in the infectivity results. Infectivity was generally better in the later experiments when plants were older.

V.4.3 SCREENING OF BRITISH ISOLATES ON DETACHED LEAVES

The difference between the number of isolates infecting detached leaves of HpilHAs and those infecting HpilGPs was striking. The screening involved only limited testing of each isolate and replication was not possible to confirm the results. The results, however, strongly indicated a difference between the localities in susceptibility to a large range of rust isolates.

V.4.4 SCREENING OF BRITISH ISOLATES ON WHOLE ROSETTES

The testing on whole rosettes of isolates that had previously infected New Zealand *H. pilosella* detached leaves resulted in the selection of a much smaller number of isolates for continued work. Only a quarter of the isolates produced infection. The most significant point was that the two top ranked isolates, BRIT12 (Innocent Railwayline, Edinburgh, UK) and BRIT21 (Slochd, UK) were, respectively, the first and third ranked isolates from the 1992 detached leaf screening. This gave weight to the reliability of the different screening methods in choosing highly infective isolates. Furthermore, isolate BRIT12 was the top ranked isolate in Section V.3.3 in terms of the proportion of HpilHOs rosettes infected.

V.4.5 INITIAL SCREENING OF IRISH ISOLATES

The result of the initial screening was to select 32 isolates, on the basis of producing sori on New Zealand *H. pilosella*, for further experiments.

V.4.6 SCREENING OF 32 IRISH ISOLATES ON DETACHED LEAVES

The experiment demonstrated that all of the isolates were capable of infecting *H. pilosella* from the two localities. As the differences between isolates, in terms of sori produced, were found to be significant, the ranking was used to select isolates for further experimentation. The top five isolates affected the vast majority of detached leaves tested (96%). These five isolates were then compared on whole rosettes and detached leaves in Section V.3.6.

V.4.7 COMPARISON OF FIVE IRISH ISOLATES ON WHOLE ROSETTES AND DETACHED LEAVES

The experiment demonstrated the ability of the Irish isolates to infect the majority of tested rosettes. The top ranked isolate, ÉIRE14, was also the top ranked isolate in Section V.3.5. The Irish isolates were noticeably more infective than the British isolates on both detached leaves and whole rosettes.

V.4.8 COMPARISON OF CONTINENTAL EUROPEAN ISOLATES

The results allowed the selection of 25 isolates for further tests. Susceptibility of rosettes to rust infection was demonstrated well by the infection of all four rosettes inoculated with isolates from the British Isles.

V.4.9 COMPARISONS BETWEEN COLLECTIONS

Isolate ÉIRE14 was the top ranked isolate, as in previous experiments, and was also the only isolate to infect a rosette from locality HpilGPs. The least significant difference was large because of the presence of many no infection values increased variance.

V.4.10 GENERAL DISCUSSION OF SCREENING AND COMPARISONS

Amongst the successfully infecting isolates, the main difference noted was between the highly infective isolates chosen from the Irish collection and isolates chosen from the other areas. Isolate ÉIRE14 achieved the highest rank for infectivity of all isolates tested. This isolate was then tested for infectivity on a range of New Zealand *H. pilosella* (Sections III.5.3 and III.5.4) and on other *Hieracium* species (Section III.7.1.c(ii)). The direct effect of ÉIRE14 isolate infection on the growth of New Zealand *H. pilosella* was also measured (Section III.9).

Screening was often limited by the processing time needed for the large number of isolates involved and therefore some good isolates may have been overlooked in the process.

The proportion of detached leaves infected was generally larger than that of rosettes infected but in several parts of the screening procedure, top ranked isolates in detached leaf work were the same as those top ranked on whole rosettes.

Because of the limited testing of the majority of British isolates, it was not clear whether an isolate not producing sori was due to genetic incompatibility of rust and

host. No resistance reactions were seen. Most testing did not result in 100% of inoculated host plants or detached leaves being infected. The highest ranked isolates produced sori on the majority of inoculated rosettes.

The top ranked isolates were from the northwest areas of rust collections, particularly Ireland and Scotland. Three Continental isolates also displayed an ability to infect a range of New Zealand *H. pilosella* in limited testing. In light of screening, 20 isolates including those most infective, (listed in Table 5.9) were deemed the best and some combination of these is likely to be the basis for application to introduce to New Zealand as biological control agents for *H. pilosella*.

Apart from those selected on the basis of the screening procedure, ÉIRE4 was selected for good spore production on *H. pilosella* ex HpilHAs (pentaploid) rosettes; BRIT32 was selected because it caused severe symptoms in the field, was from a cold area and infected some HpilGPs locality *H. pilosella*; BRIT2 was selected because of reasonable infectivity in the detached leaf screening (Section V.3.1) and because it was collected from a cold area.

Table 5.9. Source localities of selected rust isolates.

Isolate Code	Source of Isolate
ÉIRE14	SW of Slieveroe, County Kilkenny, Ireland
ÉIRE32	S of Cork, County Cork, Ireland
ÉIRE29	Riverstick, County Cork, Ireland
ÉIRE45	Upper Lake, County Kerry, Ireland
ÉIRE66	Mitchelstown, County Cork, Ireland
ÉIRE79	Durrow, County Laios, Ireland
ÉIRE39	NW of Ballylickey, County Cork, Ireland
ÉIRE13	SW of Slieveroe, County Kilkenny, Ireland
ÉIRE4	S of Bray, County Wicklow, Ireland
BRIT12	Innocent Railwayline (i), Scotland
BRIT21	Slochd I, Scotland
BRIT330	SW of West Linton, Scotland
BRIT627	NW of Langholm, Scotland
BRIT32[a]	S of Aviemore I, Scotland
BRIT51[a]	Salisbury Crags II, Scotland
BRIT53[a]	Sanna Bay, Scotland
BRIT2	Ben Nevis, Scotland
SVER341	Sävsjö, Sweden
FRAN4	SW of Verrières, France
FRAN262	Peyre Haute, France

[a] These three isolates were selected on the basis of severe rust symptoms produced on some of the experimental rosettes (e.g. severe symptoms caused by isolate BRIT53 are shown in Fig 3.17).

Chapter VI: *HIERACIUM* PATHOGENS - OTHER THAN RUST

VI.1 INTRODUCTION

In addition to rust, three further obligate fungal pathogens were observed during northern hemisphere studies on *Hieracium*. They were *Erysiphe cichoracearum*, *Entyloma* de Bary and *Bremia lactucae* Regel. Powdery mildew, *E. cichoracearum*, was experimented with by Scott (pers. comm. 1995a) in earlier work on *Hieracium* biological control (Section I.5.1.e). Scott's isolates of *E. cichoracearum* were able to infect a non target plant of another genus and therefore host specificity was investigated in the present study. The smut, *Entyloma* sp., and the downy mildew, *B. lactucae*, were also identified on *Hieracium* but were not commonly observed. The potential of the latter two pathogens as biological control agents, was assessed on the basis of disease symptoms and reports in the literature.

VI.2 RECORDS

In Europe, a range of fungal pathogens have been recorded on *Hieracium* subgenus *Pilosella*. In parts of North America, where *Hieracium* subgenus *Pilosella* spp. have naturalised, there are also a number of pathogens recorded, some of which would have been introduced with their hosts. There are no verified records of fungal pathogens on *Hieracium* in New Zealand (Pennycook, 1989; E. McKenzie, pers com).

VI.2.1 PATHOGENS OF *HIERACIUM* IN EUROPE

Some of the fungal pathogens, and one virus, recorded on *Hieracium* in Europe are presented in Table 6.1. In the present study, a survey of over 10 000 sites recorded rust on over 1300 sites and powdery mildew on 47 sites. The proportion of sites hosting rust or powdery mildew is probably higher than indicated by these records because many sites were visited during parts of the season unsuitable for growth of the pathogens. The *Entyloma* smut was only found in two of the sites visited in Europe and the downy mildew not observed at all in the field. *Ramularia filaris* Fres. var. *hieracii* Baüml., an imperfect fungus, was reported by Chevassut (1987) to be

very rare on *H. pilosella*, not having been previously recorded in France, and not recorded at all since 1907. Chevassut described the disease as weak, with only three or four leaves infected on just three rosettes.

VI.2.2 PATHOGENS OF *HIERACIUM* IN NORTH AMERICA

Records from North America are presented in Table 6.2, with data from Farr *et al.* (1989). Of the 28 fungal pathogens, most affect the common naturalised species *H. aurantiacum* (subgenus *Pilosella*). In addition, Farr *et al.* (1989) included a record of *Septoria hieracicola* on *H. piloselloides* Vill. (= *H. florentinum* All.; subgenus *Pilosella*). The smut *Entyloma calendulae* is listed by Farr *et al.* (1989) on an *Hieracium* sp. (subgenus not given). Another smut, *Entyloma compositarum* Farlow, is listed on *H. albertinum* Farr., *H. albiflorum* Hook. and *H. cynoglossoides* Arv.-Touv., which are all native North American species of the subgenus *Stenotheca*. Sell (1987) put forward a convincing argument for taxa of subgenus *Stenotheca* to be considered as separate from the genus *Hieracium*; he stated that on morphological grounds, they should best be placed in *Crepis*.

Table 6.1. Pathogens recorded on *Hieracium* spp. in Europe.

Pathogen	Common Name	Plant Part Affected	Recorded Host	Region Recorded From
Ascochyta hieraciicola[b]		leaves (presumed)	*H. villosum*	
Bremia lactucae[a,c]	downy mildew	leaves	*Hieracium* sp.	Britain
Entyloma hieracii[a,d]	smut	leaves	*H. murorum*	Ukraine
Erysiphe cichoracearum[a,b]	powdery mildew	above ground	*H. alpinum*	
Puccinia hieracii var. *hieracii*[a,b]	rust	leaves		
Puccinia hieracii var. *piloselloidarum*[a,b]	rust	leaves	*Pilosella* spp. including *H. pilosella*	
Ramularia filaris var. *hieracii*[e]		leaves	*H. pilosella*	France etc
Septoria mougeotii[e]		leaves (presumed)	*H. stoloniferum*	Britain, France, Germany
Sphaerotheca fuliginea[f]	powdery mildew	leaves	*H. pilosella*	Eastern Europe
Valdensia heterodoxa[b]			*H. boreale*	
Aster yellows virus[b]		leaves (presumed)	*H. floribundum*	

[a] Obligate parasites.
[b] Records from the Review of Applied Mycology. Plant Host - Pathogen Index to vols 1-40, 1922-61.
[c] From Francis and Waterhouse (1988).
[d] From Vánky (1990).
[e] From Chevassut (1987).
[f] From Grove (1935).

Table 6.2 Pathogens recorded on *Hieracium* spp. in North America.

Pathogen	Common Name	Plant Part Affected	Recorded Host	Region Recorded From
Ascochyta sp.	leaf spot	leaves	*H. aurantiacum*	Wisconsin
Bremia lactucae[c]	downy mildew	leaves (presumed)	*H. aurantiacum*	Wisconsin
Cercospora erechtitis		leaves (presumed)	*H. aurantiacum*	Wisconsin
Cercospora hieracii		leaves	*H. venosum*	Alaska and North Carolina
Cercospora sp.		leaves (presumed)	*H. longipilum*	
Entodesmium niessleanum			*H. albiflorum*	Washington State
Entyloma calendulae[c]	smut	leaves (presumed)	-subgenus not given-	
Entyloma compositarum[c]	smut	leaves (presumed)	*H. albertinum,*	
			H. albiflorum,	
			H. cynoglossoides[a]	
Erysiphe cichoracearum[c]	powdery mildew	leaves (presumed)	*H. aurantiacum*	Texas
Heteropatella cercosperma	leaf spot	leaves	*H. aurantiacum*	
Leptosphaeria salsolae		leaves (presumed)	*H. albiflorum.*	Washington State
Phoma exigua		leaf spot	*H. aurantiacum*	
Phoma hieracii		stem blight	*H. aurantiacum*	
Phomopsis hieracii		on living leaves	*H. aurantiacum*	
Phymatotrichopsis omnivora		root rot	*H. aurantiacum*	
Pleospora helvetica			-subgenus not given-	Washington State
Pleospora lactucicola			*H. albertinum*	Washington State
Puccinia columbiense[bc]	rust	leaves (presumed)	-subgenus not given-	
Puccinia fraseri[bc]	rust	leaves	*Hieracium* spp. including *H. scabrum*	Nova Scotia
Puccinia hieracii var. *piloselloidarum*[c]	rust	leaves (presumed)	*H. aurantiacum*	--Texas
			H. pratense[b] = *H. caespitosum*	--New Brunswick, Canada
Puccinia hieracii var. *hieracii*[c]	rust	leaves (presumed)	Subgenus *Hieracium* spp.	
Ramularia macrospora		leaves (presumed)	*H. albiflorum*	Washington State
Ramularia parva		leaves (presumed)	*H. longipilum*	Wisconsin
Ramularia taraxaci		leaves (presumed)	*H. longipilum*	Wisconsin
Ramularia sp.		leaves	*H. longipilum*	Wisconsin
Septoria hieracicola		leaves (presumed)	*H.* spp. including *H. piloselloides*	
Sphaerotheca fuliginea	powdery mildew		-subgenus not given-	Illinois
Sphaerotheca mucularis	powdery mildew		*H. albiflorum*	Washington State

[a] All three species are native North American members of the subgenus *Stenotheca* therefore distinct from subgenera *Hieracium* and *Pilosella*

[b] Record from Parmelee and Savile (1981), *H. pratense* Tausch = *H. caespitosum* Tausch.

[c] These species are obligate parasites.

VI.3 POWDERY MILDEW

The powdery mildews are very common obligate parasites that affect a wide variety of plant families. Their characteristic powdery appearance on hosts is due to the growth of mycelium on the surface of host tissue and the production of conidia. The only penetration of host tissue is by haustoria growing into epidermal cells to derive nutriment. The sexual stage involves the production of asci within cleistothecia which are produced in response to unfavourable conditions. The cleistothecia can act as overwintering structures.

Three genera of powdery mildew, *Erysiphe* Hedw. f ex DC., *Sphaerotheca* Lév. and *Leveillula* Arnaud are common on the Asteraceae. Powdery mildews of the genera *Uncinula* Lév. and *Microsphaera* Lév. are recorded on just one or two Asteraceae genera.

Most records of powdery mildew on *Hieracium* are for *Erysiphe cichoracearum.* However, there has been one record of *Sphaerotheca fuliginea* (Schlechtendahl) Poll. on *H. pilosella* in the Baltic region (Hirata, 1966 - source reference not given) and *Leveillula taurica* (Lév.) Arnaud has been recorded on *H. umbellatum* L. in Hungary (Hirata, 1966). *S. fuliginea* and *E. cichoracearum* are both common pathogens with very wide host ranges recorded and are frequently confused with each other (Yarwood, 1973).

VI.3.1 FIELD OBSERVATIONS

VI.3.1.a Introduction

Investigations into the potential of the powdery mildew included observations of field presence and effect of the fungus.

VI.3.1.b Methods

Note was taken of the presence of powdery mildew on *Hieracium* spp. during field collections of rust isolates. Ten collections of powdery mildew were made from ten

different areas of Britain. Species identification was verified by microscopic investigation of the conidia and cleistothecia.

During the field study described in Section IV.2 the level of powdery mildew was measured in each of the 236 patches investigated in the Holyrood Park area. As in the methods of Section IV.2.1.b(i), multiple regression was employed on transformed powdery mildew levels ($\log_{10}(\text{level} + 1^{-10})$) to determine which site factors significantly affected the level of powdery mildew.

VI.3.1.c Results

All ten collections of powdery mildew in this study were found to produce abundant ellipsoidal conidia in chains lacking fibrosin bodies. On that basis the specimens were identified as *E. cichoracearum*. Cleistothecia were only observed at one site, south of Aviemore, Scotland (isolate PM3). These were found to contain two asci, therefore confirming the identification as *E. cichoracearum*. The species *Sphaerotheca fuliginea* which is commonly confused with *E. cichoracearum*, has conidia with fibrosin bodies and cleistothecia containing just one ascus.

Powdery mildew was observed on approximately 0.5% of the greater then 10 000 *Hieracium* populations surveyed for rust throughout Europe. It was noted on members of subgenus *Hieracium* and also on *H. pilosella*, *H. praealtum*, and *H.* x *stoloniflorum*. Symptoms were occasionally severe on *H. pilosella* and sufficient to cause rosette death in combination with dry conditions. The pathogen was most noticeable in populations of *Hieracium* spp. on relatively dry banks.

Powdery mildew was found on rosettes of 19% of the patches surveyed in Holyrood Park. The area (site location) of the Park in which a patch was located significantly ($p=0.024$) affected the level of powdery mildew present. A model incorporating site location ($p=0.015$) and four site factors accounted for 13.2% of the variation of powdery mildew level in a patch; the four factors were soil moisture level (negative relationship; $p=0.013$), percentage rosettes infected with rust (negative relationship;

p=0.022), diameter of patch (positive relationship, p=0.025) and aspect (lower powdery mildew levels with more southerly aspects; p=0.031). The four site factors alone accounted for 11.4% of the variation in powdery mildew level.

VI.3.2 HOST RANGE

VI.3.2.a Introduction

Scott (pers. comm. 1995a) conducted host specificity testing on *E. cichoracearum* from *H. pilosella* and reported that the powdery mildew could infect the New Zealand endemic species *Kirkianella novae-zelandiae*. Host specificity testing of several isolates of *E. cichoracearum* from *H. pilosella* were tested on a range of species in this thesis.

VI.3.2.b Methods

In the present study, ten powdery mildew field isolates (Table 6.3) were tested for pathogenicity on five plants each of a selection of target and non-target species. The target plants were *H. aurantiacum* (HaurPPa), *H. caespitosum* (HcaeLCl), *H. pilosella* (HpilMJt), *H. praealtum* (HpilLCl), *H.* x *stoloniflorum* (HstoCRa) and from the subgenus *Hieracium*, *H. lepidulum* (HlepLRd) and *H. murorum* (Holyrood Park, Edinburgh, UK). The non-target plants included four New Zealand native species, *Embergeria grandifolia*, *Kirkianella novae-zelandeae*, *Microseris scapigera* and *Sonchus kirkii*, and four New Zealand adventive species, *Crepis capillaris*, *Hypochoeris glabra*, *H. radicata*, and *Taraxacum officinale*. All plants were 4-5 month old seedlings.

Table 6.3. Source localities of the ten powdery mildew isolates used in pathogenicity experiments.

Isolate Code	Locality	Grid Coordinates[a]
PM1	Innocent Railwayline, Edinburgh	NT 2772
PM2	Salisbury Crags, Edinburgh	NT 2773
PM3	S. of Aviemore, Scotland	NH 8807
PM4	S. of Aviemore, Scotland	NH 8807
PM5	Balmacara, Scotland	NG 8127
PM6	Arnside, England	SD 4679
PM7	Arnside, England	SD 4679
PM8	Dalkeith, Scotland	NT 3667
PM9	Glencorse Reservoir, Scotland	NT 2264
PM10	Braid Hills, Edinburgh	NT 2570

[a] From British Ordnance Survey maps.

Either whole plants or detached leaves (kept on 0.95% water agar) were used. Two methods of inoculation were employed. The first involved the deposition of large amounts of inoculum on leaves by brushing with *H. pilosella* leaves which hosted heavily sporulating powdery mildew. Following this dry inoculation, plants were lightly misted and kept in moist chamber for two hours. The second method involved inoculation with a 10^5 conidia ml^{-1} suspension, containing one drop per litre of 'Tween 80' wetting agent. Fresh spore suspension was applied with an atomiser in the evening to leave a moist film on all host leaf surfaces. Rosettes and leaves were then left to dry slowly overnight in glasshouse conditions (15°C dropping to 8°C). Subsequently, detached leaves were kept in covered petri dishes in the glasshouse. The inoculum was provided by isolates PM1 to PM5. Each of the five isolates was tested on each species. Isolates PM6 to PM10 were collected after the main specificity experiments and were only tested against *E. grandifolia* and *K. novae-zelandiae*.

Rosettes and leaves were monitored for powdery mildew infection. Any species infected and not from the genus *Hieracium* were investigated to ascertain the possible presence of a contaminant mildew. Conidia were taken from these plants and deposited onto powdery mildew free *H. pilosella* detached leaves on agar plates.

Conidia from any powdery mildew subsequently infecting the detached *H. pilosella* leaves were collected and deposited onto detached, powdery mildew free, leaves of the respective non-target species; these leaves were then monitored for powdery mildew infection.

VI.3.2.c Results

In infectivity studies the powdery mildew was shown to have a 100% infection rate on *H. pilosella*, both on detached leaves and on whole plants. Colonies began to produce conidia after just four days. Infection was also rapid on the leaves of *H. aurantiacum*, *H.* x *stoloniflorum* and *H. caespitosum*. Detached leaves of *H. praealtum* were very susceptible to the powdery mildew though whole plants did not display as severe symptoms as other species; *H. praealtum* flower stems appeared to be more susceptible than the leaves. *H. lepidulum* and *H. murorum* (subgenus *Hieracium*) were never observed to be infected by the powdery mildew, even after inoculations of whole plants and detached leaves.

All samples of the *Hieracium* powdery mildew consistently infected rosettes and detached leaves of *Kirkianella novae-zelandiae* and *Embergeria grandifolia*. No control leaves were infected. Reinfection of New Zealand *H. pilosella* whole plants and detached leaves was possible using powdery mildew conidia from infected *K. novae-zelandeae* and *E. grandifolia*. Conidia were then taken from those *H. pilosella* leaves and inoculated onto mildew free detached leaves of *E. grandifolia* and *K. novae- zelandiae*. All leaves developed powdery mildew, the conidia of which were again able to reinfect *H. pilosella*. This process was repeated for a total of five cycles with no apparent loss in virulence of the powdery mildew.

Powdery mildew infection was observed on *Taraxacum officinale*, one week after inoculation with isolate PM4. The powdery mildew only spread to a diameter of 0.5 cm and produced sparse chains of conidia. Inoculations with these conidia did not produce any infections on *T. officinale* or *Hieracium pilosella* and it was therefore unconfirmed that the mildew colony was from the inoculation with PM4. The colony

on *T. officinale* only lasted one month before senescing, probably demonstrating incomplete compatibility with the host.

The remaining species, i.e. *Microseris scapigera*, *Sonchus kirkii*, *Crepis capillaris*, *Hypochoeris glabra*, and *Hypochoeris radicata* were not observed to host any powdery mildew infection.

VI.3.3 GLASSHOUSE OBSERVATIONS

Epidemic spread of powdery mildew was rapid during most of the summer months on *Hieracium* collections. There was also a rapid epidemic of powdery mildew on the collections in the heated Montpellier glasshouses over the winter period. Symptoms on many plants, including most infected *H. pilosella*, were severe. A white covering of mildew mycelium covered the leaf surfaces of many plants. Plants appeared to be detrimentally affected by the infection.

Powdery mildew infections hampered the *Hieracium* rust pathogenicity work of Scott (pers. comm. 1995a) and also of this study. Powdery mildew contamination appeared to reduce host quality and possibly influenced rust infection. Continual control, as described below, was necessary from late spring to late autumn to keep host plants powdery mildew free in Edinburgh and also during the winter period in the Montpellier glasshouses.

The main powdery mildew control method was to remove contaminated leaves or leaf sections and in some cases whole plants were discarded. A severe epidemic of powdery mildew in late October in the Montpellier glasshouses possibly corresponded to the generally warmer and drier conditions there, compared to Scotland. Because of the severity of the epidemic, a fungicidal spray (Nimrod at 1%) was applied. Plants infected with stock collections of the rust were sprayed lighter than rust free plants. The spraying was only partially successful. Although the epidemic was apparently halted, powdery mildew was evident within three weeks and another epidemic soon followed despite leaf clipping. Rust pathogen experiments were still able to be conducted because of the large number of *Hieracium* plants that had been planted.

VI.3.4 EFFECT OF POWDERY MILDEW ON RUST

The powdery mildew was often found on rust infected patches of *H. pilosella* in the field. The presence of mildew infections adjacent to rust sori is shown in Fig 6.1. Although it was possible for the two to be found together, field results indicated an inverse relationship between the level of mildew and the severity of rust infection (see Section IV.2.1.d). Scott (pers. comm. 1995a), however, observed that rust infected plants in the glasshouse may have been more susceptible to powdery mildew.

VI.3.5 DISCUSSION

VI.3.5.a Field observations

The relationship between powdery mildew level and both low soil moisture level and low percentage rust infection was probably due to powdery mildew being favoured by drier conditions in contrast to rust. Traditionally, relatively dry conditions have been considered to be favourable to powdery mildew growth though there is some debate on the general applicability of this phenomenon (Wheeler, 1981).

The positive relationship with patch diameter could have been due to several factors. The greater range of environmental conditions present in larger patches and the increased chance of powdery mildew spore deposition may be responsible for the higher levels of powdery mildew. The prostrate form of *H. pilosella* affords an open environment making larger patches generally drier, potentially favouring powdery mildew growth.

The relationship with aspect was more difficult to interpret. Patches on more southerly aspects (facing the sun) had relatively lower levels of powdery mildew. *H. pilosella* patches were relatively less common on southerly aspects except where the slope was only slight and grass growth was not too tall; there was potential for the moisture of most patches to be effectively dried out by sunny conditions even if the patch did not slope towards the sun. For this reason the relationship observed between aspect and powdery mildew level does not necessarily conflict with the fact that powdery mildew growth might be favoured by less humid conditions. The

negative relationship with southerly aspect could have been confounded with the more extreme conditions on steeper slopes (which were mainly on south eastern to south western aspects).

VI.3.5.b Host range

Scott (pers. comm. 1995a) (Section I.5.1.e) found that powdery mildew could cross from *H. pilosella* to the New Zealand native *Kirkianella novae-zelandiae*. In the present study, *K. novae-zelandeae* and also *Embergeria grandifolia* were infected by *Hieracium* powdery mildew. Scott (pers. comm. 1995a) found that most other species of the Lactuceae were not infected by the powdery mildew isolates, though occasional small colonies of powdery mildew were noted on some *Cichorium endivia* L., *Lactuca sativa* L. and *Taraxacum officinale* Wigg.. Small colonies of powdery mildew were also noted on two Asteraceae species from different tribes, *Achillea millefolium* L. (yarrow) and *Chrysanthemum maximum* Ramond (chrysanthemum). Conidia of these non-target infections were not inoculated on to *H.* pilosella to determine if the powdery mildew was a contamination from the atmosphere. In the present study, reinfection of *H. pilosella* confirmed that the powdery mildew on *K. novae-zelandeae* and *E. grandifolia* was of the same form as that infecting *Hieracium* subgenus *Pilosella.*

Blumer (1922) experimented with *E. cichoracearum* from *H. pilosella* (subgenus *Pilosella*) and from *H. murorum* (subgenus *Hieracium*). Powdery mildew from *H. pilosella* was found by Blumer to infect all three species of subgenus *Pilosella* that he tested, and not infect any of the 15 tested taxa of subgenus *Hieracium.* With the isolate from *H. murorum*, infection was produced on all but two of the 15 subgenus *Hieracium* taxa and not on any of the three subgenus *Pilosella* species. These results agree with the negative results of inoculating *H. lepidulum* (subgenus *Hieracium*) with *E. cichoracearum* from *H. pilosella* in the present glasshouse experiments. In the present study, the field occurrence of powdery mildew on subgenus *Hieracium* species and *H. pilosella* adjacent to each other may have been coincidental. The ability for cross-infection of powdery mildew between these plants was not tested. Blumer (1922) reported conidial sizes and shapes of several isolates of *E.*

cichoracearum, but no apparent significant difference between isolates from the different subgenera *Hieracium* hosts were noted.

Hasan (1974b) found that an isolate of *E. cichoracearum*, proposed for potential biological control of *Chondrilla juncea*, demonstrated suitable host specificity. In the cases where non-target species became infected, cross infection of the conidia back onto *C. juncea* did not give rise to infection; the plants of the infected species were resistant to subsequent inoculation attempts. It was therefore concluded that the origin of the powdery mildew on non-target species was from contaminating spores from outside the glasshouse. In all, Hasan tested twenty members of the Asteraceae including eight species of Lactuceae, *Cichorium endivia*, *Crepis taraxacifolia* Thuill., *Helminthia echioides* (L.) Gaert., *Lactuca sativa* (3 cultivars), *Picris hieracioides* L., *Sonchus asper* All., *Taraxacum officinale*, and *Urospermum dalechampii* Desf.. Members of 21 other plant families were also included in host specificity testing. Hasan (1980) reported that it was not yet possible to introduce *E. cichoracearum* to Australia for the control of *C. juncea* because the stability of host specialisation was in some doubt.

VI.3.5.c Glasshouse observations

The spread and severity of powdery mildew in the glasshouses was dramatic. In terms of pathogenicity, the pathogen displayed good potential as a biological control agent of *Hieracium*. Fig 6.2 shows a moderate powdery mildew infection on glasshouse grown *H. pilosella* leaves.

The results presented here for *Hieracium* powdery mildew confirmed the ability of the powdery mildew strains tested to infect other species. It seems that most species, even in the Lactuceae, are, however, not susceptible to infection. Although there is obviously a degree of host specificity present in various strains of *E. cichoracearum*, it is not as great as that present in the rust *P. hieracii* var. *piloselloidarum*.

VI.3.5.d Powdery mildew in New Zealand

There are many species of powdery mildew in New Zealand including *Erysiphe cichoracearum*. This species has been recorded on a variety of hosts in the country, including a wide variety of members of Asteraceae and also 16 other families (Boraginaceae, Brassicaceae, Caryopyllaceae, Crassulaceae, Cucurbitaceae, Geraniaceae, Gesneriaceae, Lamiaceae, Malvaceae, Myrtaceae, Papaveraceae, Plantaginaceae, Scrophulariaceae, Solanaceae, Valerianaceae and Violaceae) (Boesewinkel, 1979; Pennycook, 1989). There have, however, been suggestions that there are several different forms of *E. cichoracearum*, perhaps with one form being specific to the Asteraceae (Hammett, 1977) and even within such forms there appear to be strains which are specific to a smaller range of species. Of the Lactuceae, three adventive species are recorded as host to *E. cichoracearum*, namely *Picris echioides* (oxtongue), *Taraxacum officinale* (dandelion) and *Tragopogon porrifolius* (salsify) (Pennycook, 1989). There have, however, been no records of powdery mildew on *Hieracium* in New Zealand. This may indicate a lack of *E. cichoracearum* strains or *formae speciales* pathogenic to New Zealand *Hieracium* or that the strains have not had suitable opportunity to cause an observed infection on *Hieracium*.

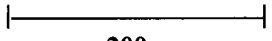
VI.3.6 PROSPECT FOR BIOLOGICAL CONTROL WITH POWDERY MILDEW

Powdery mildew demonstrates high infectivity and sufficient virulence for biological control of *Hieracium*, particularly *H. pilosella*. The pathogen would have the ability to grow in the drier zones of *Hieracium* infestation. The negative correlation between rust and powdery mildew in the field could simply reflect the fact that the optimum conditions for powdery mildew disease are generally drier than those for rust. However, the lack of perceived strict host specificity may be a problem. An argument for introduction despite the effects on at least two native species related to *Hieracium* would probably require evidence that the benefit of biological control outweighs the potential detrimental effect of the continued spread of *H. pilosella* on the endemic species populations, an indirect effect of the powdery mildew.

Fig 6.1 **Powdery mildew - micrograph**

SCANNING ELECTRON MICROGRAPH (x 130)

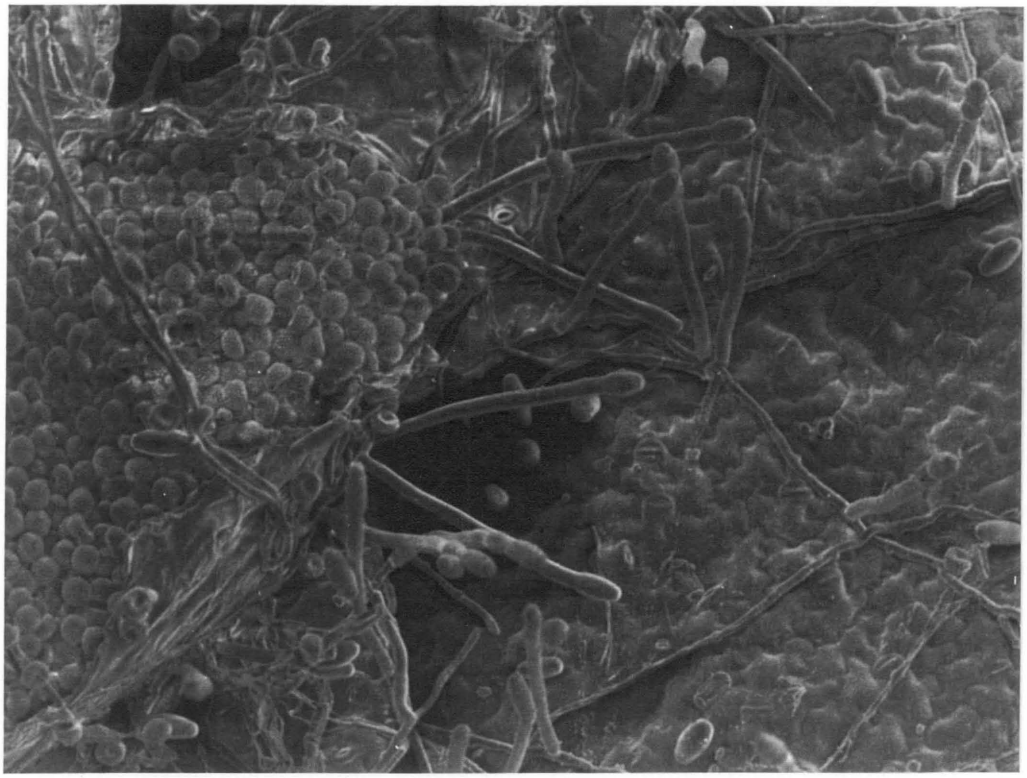
A network of powdery mildew mycelia (*Erysiphe cichoracearum*) growing in close proximity to a rust (*Puccinia hieracii* var. *piloselloidarum*) on the surface of an *H. pilosella* leaf. Conidiophores of powdery mildew protrude and produce conidia.

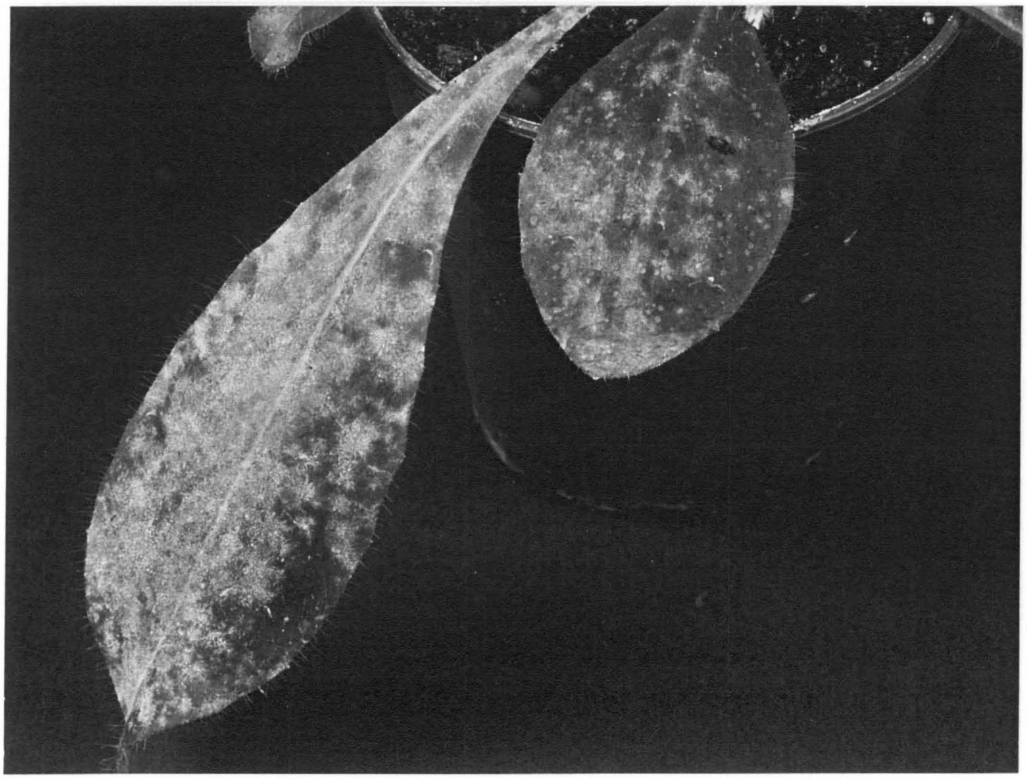


200μm

Fig 6.2 **Powdery mildew infection**

H. pilosella leaves infected with powdery mildew (*E. cichoracearum*). It was common for most leaves of powdery mildew infected plants to become covered with mycelium.





The risk that the host range of the powdery mildew may extend beyond the two native species and *Hieracium* may be less significant, given that *Erysiphe cichoracearum* is already present in New Zealand on a wide variety of hosts including three species of the Lactuceae. The native Lactuceae species, *Kirkianella novae-zelandeae*, growing in the high country, is at risk from continued *Hieracium* expansion. *Embergeria grandifolia* is endemic to the Chatham Islands and therefore in its natural environment is isolated from the high country *Hieracium* infestations and presumably from heavy sources of inoculum.

VI.4 DOWNY MILDEW

VI.4.1 INTRODUCTION

The downy mildews are mostly foliage blights that cause damage to a variety of hosts. Some downy mildew species can spread very quickly and in suitable conditions can cause severe damage to their host (Agrios, 1978). The pathogens belong to the family Peronosporaceae of the Oomycetes. Most downy mildews are obligate parasites. The genera of downy mildew reported on Lactuceae hosts include *Bremia* Regel, the most common, and *Peronospora* Corda and *Plasmopara* Schroet., the latter two recorded on two species of the Lactuceae each. *Bremia* is mainly represented by one species, *B. lactucae*, which affects only species of the Asteraceae, including lettuce *Lactuca sativa* and at least 18 other genera of the Lactuceae. Only *Bremia lactucae* is recorded on *Hieracium*.

VI.4.2 OBSERVATIONS

B. lactucae was identified on New Zealand *H. pilosella* growing in Edinburgh glasshouses, possibly having been inadvertently collected with one of the *Hieracium* plant collections from the field in Britain. The pathogen infected older leaves of the rosettes producing a mousy-white down. The observed infections did not appear to be severe. The narrow emergent sporophore branches with distinctly swollen tips and a small number of peripheral sterigmata that bear the unornamented conidia.

VI.4.3 DISCUSSION

There was no evidence to suggest that the downy mildew fungus would provide effective biological control of *Hieracium* in New Zealand. Downy mildew severity relies greatly on the maintenance of a film of water on the plant surface and consequently the disease is favoured by damp and cool to warm. Any effects of the pathogen would thus most likely be seen in the spring and autumn. The disease was not observed on *Hieracium* during any field work throughout Europe, though it may have been overlooked due to lack of sporulation. *Bremia lactuceae* commonly occurs on lettuce and other species so host specificity of *Hieracium* downy mildew would require careful investigation.

VI.5 ENTYLOMA SMUT

VI.5.1 INTRODUCTION

There are around 150 accepted species of *Entyloma* infecting plants of 30 families (Vánky, 1987) and a number of species of *Entyloma* have been recorded in New Zealand (Pennycook, 1989), including four species on Asteraceae hosts, *E. calendulae* (Oudemans) de Bary, *E. compositarum* , *E. dahliae* H.&P. Sydow and *E. picridis* Rostrup. Two of the species have been recorded on adventive Lactuceae hosts; *E. picridis* on *Picris echiiodes*, and *E. compositarum* on *Crepis capillaris* and *Lapsana communis*. *E. compositarum* is also recorded on *Senecio minimus* Poiret from the subfamily Asteroideae.

Entyloma taxonomy is hindered by a limited number of distinguishing morphological characters. *Entyloma* taxa which infect members of the Asteraceae are commonly treated in the species *E. compositarum* Farlow and all specimens of this species seem morphologically similar irrespective of host range. The limitation of *Entyloma* species to just one host plant family is based on the proposal of Fischer (1953) and Fischer and Shaw (1953). Host specialisation is very important in *Entyloma* taxonomy (Durán, 1973) and there may be species described for particular host genera, e.g., *Entyloma hieracii* H. & P. Sydow ex Ciferri on *Hieracium* in Europe.

Entyloma species reproduce by forming teliospores but species often have anamorphs (*Entylomella* von Höhnell) also, which reproduce by the production of conidiophores protruding from stomata (Vánky, 1987). The conidiophores bear asexual, filiform, curved, hyaline conidia.

VI.5.2 FIELD AND GLASSHOUSE OBSERVATIONS

During cooperative field work with this thesis, S. Hasan (pers. comm.) found an *Entyloma* smut disease on *H. pilosella* plants near Puigcerda in the Spanish foothills of the Pyrenees. The specimen fits the description of *E. compositarum* by Durán (1973). Symptoms began as yellow patches on leaves. Sori with an associated brown colour developed in the centre of these patches and produced teliospores. Teliospores remain within the leaf tissue, unless physically disturbed, until leaf senescence. Several teliospores were observed after germination to produce basidia with four primary sporidia.

The smut was only observed at one site. However, S. Hasan (pers. comm.) observed smut symptoms on several plants of a collection of *H. pilosella* from Lourdes, France, transplanted into a glasshouse.

VI.5.3 EFFECT OF *ENTYLOMA* ON RUST

Entyloma was observed on the same leaves as actively sporulating rust in both field and glasshouse conditions. The rust did not appear to restrict smut infection. The development of the smut reduced the available space for rust infection and rust sori did not continue producing spores after senescence of leaf tissue caused by the smut.

VI.5.4 DISCUSSION

The rarity of smut incidence during field collections may have been due to the timing of surveys. *Entyloma* symptoms may be more obvious in autumn than spring and early summer.

VI.5.5 PROSPECT FOR BIOLOGICAL CONTROL WITH *ENTYLOMA*

Entyloma smut shows some potential for biological control but will depend on host specificity which has not been investigated. *Entyloma* is recorded on several close relatives of *Hieracium*, as well as on the subgenus *Hieracium*. Symptoms appeared to be reasonably severe on infected plants transplanted to the glasshouse. Smut infection was not observed to cause death of any *Hieracium* rosettes.

The epidemiology of *Hieracium* smut may be limited by a slow turnover of generations. The fact that teliospores remain within leaf tissue until senescence or physical disruption would also lower the rate of spread of the pathogen. In the field, only one patch was observed to have any symptoms, even though *H. pilosella* was very common in the vicinity of the infected patch. This may indicate a low transmissibility or that symptoms were yet to develop from resident infections on the other patches. The only record of weed biological control with an *Entyloma* sp. is *Entyloma compositarum* used effectively to control pamakani weed (*Ageratina riparia* (Regel). R. King et H. Robinson) in Hawaii (Trujillo *et al.*, 1988), however, this tropical smut produced conidia which were used for biological control. Conidia were not observed on the *Hieracium* smut.

VI.6 GENERAL DISCUSSION: BIOLOGICAL CONTROL WITH PATHOGENS OTHER THAN RUST

There are a large number of fungal pathogens recorded on *Hieracium* species in Europe and North America. The variety of pathogens recorded on naturalised subgenus *Pilosella* spp. in North America contrasts with the absence of these pathogens in New Zealand. The presence of *P. hieracii* rust on naturalised species of the Lactuceae in New Zealand (Section I.4.2.b) further contrasts with the absence in this country of the variety that infects subgenus *Pilosella*.

The powdery mildew shows much pathogenicity on *H. pilosella* but the isolates experimented with were not strictly host specific. The smut and downy mildew did not appear very promising because of their perceived low frequency in the field. The life cycle of the smut may also restrict biological control potential. Further work on these pathogens would require an investigation into the life cycle and chance for asexual sporulation of the smut, and surveys on the presence and effect of the pathogens.

There are a variety of non-obligate pathogens that may have potential as fungal biological control agents. In preliminary studies of the mycoherbicidal control of *H. pilosella* and *H. praealtum*, Gedyes and Harvey (1994) found that inundative application of *Sclerotinia sclerotiorum* (Libert.) de Bary caused senescence of all leaves of rosettes of *H. pilosella* in the glasshouse (though new rosettes occasionally formed from the base of affected rosettes). Mycoherbicidal control of this nature would probably not be applicable to the control of *Hieracium* on a very large scale but in specific scenarios of a smaller scale the method may be of value.

Chapter VII: HIERACIUM TAXONOMY

Taxonomical studies were undertaken to establish a knowledge of the variation present in New Zealand *Hieracium* taxa and how this relates to rust pathogenicity. The methods used were chromosome analysis to identify ploidy levels (Section VII.1), and isozyme electrophoresis to investigate variation in enzyme forms (Section VII.2).

VII.1 CHROMOSOME ANALYSIS

VII.1.1 INTRODUCTION

Many European studies have reported on the presence and ecological significance of ploidy levels (no. of sets of chromosomes per cell) of *Hieracium* taxa. A summary of this European knowledge is presented in Section I.3.6. As an aid to understanding the genetic range within *Hieracium* species in New Zealand, a survey was carried out to determine the ploidy levels present (Section VII.1.3). Ploidy levels were also ascertained for several *H. pilosella* and other *Hieracium* spp. collections from the British Isles (Section VII.1.6).

VII.1.2 METHODS

Chromosome counts were made from root tips of the *Hieracium* plants. Selected plants were grown in optimum conditions for growth with particular attention that they were not water stressed. Plants were grown in a mixture of 1 part John Innes No.2 potting compost : 1 part sphagnum moss peat in individual 390 cm^3 pots. The greatest numbers of healthy root tips suitable for cytology were found when a young or transplanted rosette's roots had just reached the base of a plant pot. The plant pot was removed from the potting mix which was held together by roots. Suitable root tips which were active, white and with no visible sign of stress were selected. Root tips exposed rather than from within the potting mix were chosen as these were easier to clean and provide better chromosome spreads due to the lesser amount of soil

particles. The root tips were transferred with forceps to a petri dish of distilled water; thoroughly cleaned of soil particles; placed in sealed bijou bottles filled with a pretreatment solution of saturated α-bromo napthalene to halt mitosis; and incubated in darkness at room temperature for at least two hours. Longer periods of incubation, up to three hours, were used if greater contraction of the chromosomes was desired.

After pre-treatment the root tips were dabbed dry on clean filter paper; rinsed three times in distilled water in a large watch glass; dabbed dry on filter paper again; and placed in a fresh fixative solution of 3 parts absolute alcohol to 1 part propionic acid, for at least 15 minutes. Root tips were still in good condition after six months in fixative.

The cold hydrolysis Feulgen technique, described below, was employed for preparing most chromosome spreads. The technique was optimised for *Hieracium* from the methods of Dr K. Jong (pers. comm.) based on Fox (1969). Nine further counts from New Zealand *H. pilosella* were made using the method of Mahanty (1970).

Hydrolysis: Root tips were removed from the fixative; rinsed in three changes of distilled water in a watch glass; and hydrolysed in 5N HCl for 45 minutes at room temperature, in watch glasses agitated several times.

Staining: Root tips were gently shaken in distilled water for one minute to remove the acid; then blotted dry; transferred to Feulgen's reagent for one to two hours in a sealed bijou bottle kept in darkness at room temperature; rinsed in tap water for a few minutes; and washed in three changes of fresh SO_2 water (5 ml 10% potassium metabisulphite, 5 ml N HCl, 100 ml distilled water) with five minutes for each change.

Mounting: Densely stained portions of the root tips were cut on to a cleaned microscope slide in to a small drop of counterstain (2% propionic-orcein, adapted from the aceto-orcein method of M. Johnson (pers. comm.)); the selected portion from one root tip was macerated on its own slide using the tip of a broken needle;

maceration was continued until a single cell spread could be achieved; a clean No. 1 square coverslip was carefully placed over the specimen, avoiding air bubbles; and the slide was then placed between two thicknesses of filter paper and a gentle pressure was applied without sideways movement of the coverglass.

Enhancement: Several methods were employed to enhance the spread of the chromosomes. Slides were heated gently over a spirit-burner to evaporate some of the liquid and thus flatten the specimen. Slides with good specimens were sealed with rubber solution around the coverslip. Slides could be kept in the freezer overnight and then, after removing the rubber solution, gently heat treated and resealed.

Observation and recording techniques: Most records were made using video prints and some photographs were taken. For a documented count, at least five definite chromosome counts were required from each of five slides.

Permanent slides: (Dr K. Jong pers. comm.)- A steel block was placed in liquid nitrogen and then kept in polystyrene. A slide was placed on the block to freeze. A rounded scalpel was then used to separate the coverslip. The slide and cover slip were then quickly placed in alcohol for two minutes then transferred to fresh alcohol for a further two minutes. Slide ends were then blotted to remove excess alcohol. A fresh coverslip was placed over the specimen area of the slide and a small amount of Euparol was applied to the side of coverslip and allowed to penetrate and cover the specimen. If part of the specimen was present on the original coverslip, the coverslip was placed on a cleaned slide and also sealed with Euparol.

VII.1.3 PLOIDY LEVELS OF NEW ZEALAND *HIERACIUM* TAXA

VII.1.3.a Introduction

The aim was to identify whether variation in ploidy level exists within any of the *Hieracium* species present in New Zealand. The ploidy levels and variation identified could then be taken into account in terms of knowledge of the species' biology and the implications for biological control.

VII.1.3.b Methods

H. pilosella was sampled from 34 localities throughout New Zealand. For an initial pilot study of nine localities, rosettes were transplanted into glasshouse conditions. For all other locations, *Hieracium* seed was taken, at random, from many plants within an area of 0.25 hectares (at several of these localities, other species were similarly sampled). Plants were grown in a mixture of 1:1 John Innes No.2 potting compost: sphagnum peat. Locations of the collections are given in Table 2.6.

VII.1.3.c Results

There were no detected incidences of aneuploidy, i.e. departures from strict multiples of the base number. Therefore all chromosome counts obtained were the base number of nine multiplied by the factor of ploidy, such that a tetraploid (4X) had 36 chromosomes and a pentaploid (5X) had 45. The ploidy levels found ranged from triploid (3X) to hexaploid (6X).

The chromosome counts are summarised in Table 7.1. The chromosome morphology was distinct, including the presence of satellites, apparently on one chromosome per ploidy level.

Table 7.1. Frequency of ploidy levels in 49 New Zealand *Hieracium* collections.

Taxa	New Zealand Ploidy Level			
	3X	4X	5X	6X
Subgenus *Pilosella*				
H. pilosella			33	1[a]
H. praealtum				
subsp. *thaumasium*		3	2[a]	
subsp. *praealtum*			1	
H. caespitosum				
subsp. *caespitosum*		1[a]	2[a]	
H. aurantiacum				
subsp. *carpathicola*	1[ab]	1[a]		
H. x *stoloniflorum*				3[a]
Subgenus *Hieracium*				
H. lepidulum	1[a]			

[a] First record of these cytotypes in New Zealand.
[b] Ross Bicknell (pers. comm.)

All but one of the *H. pilosella* collections were exclusively pentaploid. The exception was that from the Haldon Station (HpilHAs) locality. Here, five out of the eleven seedlings sampled were hexaploid, the rest pentaploid. The subspecies identifications for *H. pilosella* are given in Table 2.6. Most of these New Zealand collections matched subsp. *micradenium* on the basis of glandular hairs on the involucral bracts. However, it is important to note that some pentaploid specimens also possessed a very small proportion of simple eglandular hairs. New Zealand pentaploid *H. pilosella* produced some viable pollen as judged by germination on 1% water agar augmented with 10% sucrose.

H. praealtum from three localities (HpraCRa, HpraLRu and HpraTWo) was tetraploid whilst the samples from the other three locations (HpraMJt, HpraMJs and HpraLCl) were pentaploid. All of the three tetraploid collections and two of the pentaploids matched subsp. *thaumasium* (Peter) P.D. Sell whereas the pentaploid HpraLCl collection matched subsp. *praealtum*, according to the key in the Flora Europaea (Sell and West, 1976).

The two collections of *H. caespitosum* both matched subsp. *caespitosum*, the only subspecies recorded in New Zealand by Garnock-Jones (in Webb *et. al.*, 1988). The HpraMJt *H. caespitosum* collection was pentaploid. Out of five rosettes sampled from the HpraLCl *H. caespitosum* collection, three were pentaploid and two tetraploid.

H. x *stoloniflorum* from three localities (Mt John, Lake Tekapo and Craigieburn) was hexaploid. A single population of *H. aurantiacum*, from Porter's Pass, which matched subsp. *carpathicola*, was tetraploid. *H. lepidulum* from Lake Coleridge was triploid.

Chromosome spreads of five *Hieracium* species are shown in Figs 7.1 to 7.5 with ploidy levels ranging from triploid (2n=27) to hexaploid (2n=54).

Fig 7.1 (left) **Chromosome analysis *H. caespitosum***
LIGHT MICROSCOPE (x 100)
ex Lake Clearwater, New Zealand (HcaeLCl).
2n = 45
3 hours α-bromonapthylene pretreatment
|————————|
20μm

Fig 7.2 (right)**Chromosome analysis *H. lepidulum***
LIGHT MICROSCOPE (x 100)
ex Hanmer Springs, New Zealand (HlepCRa).
2n = 27
3 hours α-bromonapthylene pretreatment.
|————————|
20μm

Fig 7.3 (left) **Chromosome analysis *H. pilosella***
LIGHT MICROSCOPE (x 100)
ex Haldon Station, New Zealand (HpilHas)
2n = 45
3 hours α-bromonapthylene pretreatment.
|————————|
20μm

Fig 7.4 (right)**Chromosome analysis *H. praealtum***
LIGHT MICROSCOPE (x 100)
ex Lake Clearwater, New Zealand (HpraLCl)
2n = 36
24 hours (ca. 4.5^0C) α-bromonapthylene pretreatment.
|————————|
20μm

Fig 7.5 **Chromosome analysis *H.* x *stoloniflorum***
LIGHT MICROSCOPE (x 100)
ex Mount John, New Zealand (HstoMJw)
2n = 54
3 hours α-bromonapthylene pretreatment.
|————————|
20μm

VII.1.3.d Discussion

The ploidy levels of *H. praealtum*, *H. caespitosum*, *H. aurantiacum*, *H.* x *stoloniflorum* and *H. lepidulum* in New Zealand are reported here for the first time. The hexaploid *H. pilosella* is also a first record for New Zealand. These ploidy levels are significant for the autecology of the taxa. They also provide a basis for any further intensive investigation of *Hieracium* taxonomy and variation in New Zealand. Perhaps the most significant finding is of infraspecific variation in ploidy level in New Zealand *H. pilosella*, *H. praealtum* and *H. caespitosum*.

R. Bicknell (pers. comm.) investigated the chromosome number of two populations of New Zealand *H. aurantiacum*, which he found to be triploid. This contrasts with the tetraploid result from the only population of *H. aurantiacum* studied in the present investigation. Only a few sites were investigated of *H. caespitosum* (2 sites) and *H.* x *stoloniflorum* (3 sites) and evidently there were insufficient samples to show whether variation of ploidy level exists in these taxa in New Zealand.

The absence of aneuploidy (deviation from multiples of the base chromosome number), could be expected since in the literature the incidence of aneuploidy in field collected *Hieracium* is low (see Section I.3.6).

Morphological variation in the chromosome morphology including satellites, may be useful for future taxonomic work.

VII.1.3.d(i) *H. pilosella*

The only previous records of New Zealand *Hieracium* ploidy levels were those of Makepeace (1981) for *H. pilosella*. Makepeace collected from 31 sites throughout New Zealand, all pentaploid. He collected three ramets of one clone from each site. A further 34 localities were investigated in the present study. Of these, 25 localities were sampled as seed from a wide area, and therefore the individuals counted were not likely to represent ramets of a single clone.

The hexaploid *H. pilosella* found at Haldon Station may have been introduced from Europe where this ploidy level is widely present. The possibility that the hexaploid was produced spontaneously in New Zealand from successful pollination of a pentaploid *H. pilosella* needs consideration in view of some published literature on *H. pilosella*. Although Gadella (1987) found no evidence of sexual seed production in pentaploid *H. pilosella* field collections, he could produce some sexual pentaploid progeny from sexual tetraploid *H. pilosella* pollinated by apomictic pentaploid *H. pilosella*. Some progeny from the sexual pentaploid were aneuploid, the pentaploid evidently contributing two genomes plus an incomplete set of chromosomes. However, Gadella found that a large proportion of the embryo sacs were provided with an euploid chromosome number (either diploid or triploid). Therefore, if a small degree of sexual capacity exists in New Zealand pentaploid *H. pilosella*, it could be possible for eu-triploid embryo sacs to be formed and be pollinated by triploid pollen grains resulting in hexaploid progeny. The hexaploid is not likely to have arisen in New Zealand from an unreduced apomictic pentaploid embryo sac pollinated by haploid pollen (n=9) since the diploid and triploid *H. pilosella* cytotypes (capable of producing haploid pollen) are uncommon in Europe (Section I.3.6) and not known to be present in New Zealand.

The hexaploid has a similar morphology to the pentaploid in New Zealand and differences in autecology are possibly less than those between tetraploid and pentaploid, though this requires further study. In Europe hexaploid *H. pilosella* representatives have been shown to have greater stolon lengths than the pentaploid or the tetraploid (Gadella, 1991). The breeding mechanism of the New Zealand hexaploid is unknown. Hexaploid *H. pilosella* in Europe has been described both as apomictic (Turesson and Turesson, 1960) and sexual (Gadella, 1992). Gadella (1991) also reported that hexaploid *H. pilosella* from mountainous areas of Europe reproduced sexually and some plants from lowland areas reproduced apomictically.

Morphological variation exists in New Zealand *H. pilosella* but the proportion of this due to genetic variation, as opposed to plasticity of characters, requires further investigation. Preliminary isozyme electrophoretic studies have demonstrated the

existence of genetic variation in New Zealand pentaploid *H. pilosella* (pers. obs.). Based on involucral bract hairs, most *H. pilosella* specimens were identified to subspecies level, but these identifications were not given much weight in this study. The majority of New Zealand *H. pilosella* plants match subsp. *micradenium*, though specimens matching subsp. *pilosella* and *trichosoma* have also been recorded, other specimens are difficult to place in any of these subspecies or approach other subspecies (Garnock-Jones in Webb *et al.*, 1988, p. 330). The New Flora of the British Isles (Stace, 1991) describes the subspecies as no more than varieties and as only partially or not at all geographically separated. The variation in involucral bract hairs does appear to be plastic to some extent in New Zealand *H. pilosella*. Many specimens grown from seed of rosettes which matched subsp. *micradenium* (having had no simple eglandular hairs on the involucral bracts) have produced involucral bracts with small amounts of simple eglandular hairs. The presence of genetic differences between the reported subspecies has not yet been demonstrated in New Zealand. Overall the genetic variation found in New Zealand *H. pilosella* is a small subset of that in Europe in terms of both ploidy level and morphological (i.e. morphological subspecies - Section I.3.1.e) forms. The existence of viable pollen from New Zealand *H. pilosella* showed that the cytotype has the potential to be a pollen donor in a sexual cross.

VII.1.3.d(ii) *H. praealtum*

H. praealtum is represented by at least two subspecies in New Zealand. Most New Zealand specimens match the Flora Europaea description of subsp. *thaumasium* on the basis of involucral bract hairs (Sell and West, 1976). Such specimens are present here as tetraploid and pentaploid. A sample of subsp. *praealtum* HpraLCl was pentaploid. The subsp. *praealtum* is recorded as tetraploid and pentaploid in Europe, these being the only ploidy levels recorded for the species as a whole. There are no chromosome counts from Europe specifically ascribed to subsp. *thaumasium*.

VII.1.3.d(iii) *H. caespitosum*

The *H. caespitosum* populations present in New Zealand fit the description of subsp. *caespitosum*. The New Zealand pentaploid chromosome count agrees with that

reported for the subspecies in Europe. The tetraploid counts indicate genetic variation within one site (HcaeLCl). *H. caespitosum* as a whole has records in Europe ranging from diploid to pentaploid. Gadella (1992) worked with a tetraploid apomictic *H. caespitosum* (subspecies unreported) that produced viable pollen resulting in crosses with sexual tetraploid *H. pilosella*. Gadella reported *H. caespitosum* subsp. *caespitosum* as reproducing apomictically. The work of Skaliñska and Kubieñ (1972) on tetraploid *H. caespitosum* (as *H. pratense* Tausch) suggested facultative rather than obligate apomixis.

VII.1.3.d(iv) *H. aurantiacum*

The single clone of *H. aurantiacum* (HaurPPa) sampled in this study was tetraploid. R. Bicknell (pers. comm.) worked with another clone of *H. aurantiacum* (from North Otago) and found it to be triploid. Bicknell found the triploid to be a facultative apomict. After inserting a genetic marker into the plant, Bicknell examined subsequent progeny for inheritance of the marker gene. A small percentage (ca. 2.5%) of seedling progeny raised *in-vitro* were found to lack the marker gene, indicating probable meiotic recombination in around 5% of embryos. In Poland, Skaliñska (1967, 1971) found both tetraploid and pentaploid *H. aurantiacum* to be apomictic, but she found that the apomictic tetraploid also displayed a small degree of sexual reproduction.

Skaliñska (1971) reported that the sexual embryo sac of pentaploid *H. aurantiacum* died at an early stage. She presented field evidence of apomictic embryo sacs of pentaploid *H. aurantiacum* apparently receiving fertilisation which could be provided by haploid pollen from a neighbouring subgenus *Pilosella* taxon, *H. auricula* L. (= *H.* x *floribundum* Wimmer & Grab.), resulting in a hexaploid hybrid (Skaliñska, 1967). In glasshouse experiments Skaliñska (1971) demonstrated that unreduced embryo sacs (assumed by Skaliñska to be apomictic) could indeed receive pollination, in this case diploid pollen from tetraploid *H. aurantiacum*. Two seedlings, out of the total of ten progeny examined, were hexaploid. A pentaploid pollinated by a hexaploid also gave rise to a daughter with an aneuploid chromosome number of 2n=38. Skaliñska interpreted this seedling as being a result of a meiotic embryo sac escaping abortion

and developing parthenogenetically. The seedling showed weak growth and died before any floral initiation.

The ratios of fertile to sterile pollen grains was found by Delcourt (1977) to differ between ploidy levels of *H. aurantiacum* in France. Delcourt found tetraploids produced 76% fertile pollen, pentaploids 42%, and hexaploids 68% (the method of Hrishi and Muntzig was employed to test fertility but this method is not elaborated on by Delcourt).

VII.1.3.d(v) *H.* x *stoloniflorum*

In subgenus *Pilosella*, interspecific hybrids are often allopolyploid, with each parent donating a fraction of the number of its chromosome sets or, less commonly, the entire unreduced number. The origin of the hexaploid New Zealand *H.* x *stoloniflorum* hybrids is uncertain although the ploidy level itself provides some clue. *H.* x *stoloniflorum* is common in Europe and may have been introduced to New Zealand. Only reports of pentaploid and an aneuploid (2n=46) *H.* x *stoloniflorum* are found in European literature, but there have been few recorded studies so an undetected hexaploid cytotype may exist in Europe. Alternatively, hybridisation may have occurred in New Zealand, given the ploidy levels of New Zealand plants and the likely presence of some sexual reproduction in the triploid and tetraploid *H. aurantiacum* (see discussion of *H. aurantiacum*). In Europe, pentaploid apomictic *H. pilosella* produces predominantly diploid or triploid pollen and hexaploid *H. pilosella* produces triploid pollen and egg cells (Gadella, 1987). *H. aurantiacum* could possibly produce triploid or tetraploid unreduced embryo sacs from triploid and tetraploid parents respectively, and thus fulfil the complement of a hexaploid hybrid. Alternatively, an unreduced pentaploid *H. pilosella* gamete (shown to be possible in European pollen by Gadella, 1987), or an unreduced embryo sac (such as reported in *H. aurantiacum* by Skalinska, 1971), would require a haploid gamete partner which may be possible from a triploid *H. aurantiacum* parent.

An hexaploid would not be the most expected result of crosses between the ploidy levels so far identified in New Zealand *H. aurantiacum* and *H. pilosella*. These two

species may well be represented in New Zealand by further ploidy levels, especially so given that the only two populations of New Zealand *H. aurantiacum* so far sampled were of different ploidy levels.

Hexaploid *H.* x *stoloniflorum* could have a significant degree of sexual capacity because of the even number of chromosomes.

VII.1.3.d(vi) Subgenus *Hieracium*

H. lepidulum was the only taxon of subgenus *Hieracium* included in the New Zealand investigation. The triploid count was the first known record for this species in any country. A chromosome count may, however, exist in European literature under a different binomial due to the complicated taxonomy of this subgenus. *H. lepidulum* is assigned to the *H. vulgatum* Fries. group, which has a triploid count recorded by Sell and West (1976). The triploid cytotypes are expected to produce seed by obligate apomixis. Pollen produced by two New Zealand *H. lepidulum* plants was non-viable (pers. obs.).

European chromosome counts exist for two other taxa of subgenus *Hieracium* found in New Zealand. *H. sabaudum* is recorded as triploid and *H. murorum* is recorded as both triploid and tetraploid (Sell and West, 1976).

VII.1.4 APOMIXIS - OBLIGATE OR FACULTATIVE ?

Apomixis is facultative in tetraploid *H. aurantiacum* (Skaliñska, 1971; R. Bicknell pers. comm.). Bicknell also demonstrated a small degree of apparent sexual crossing in New Zealand triploid *H. aurantiacum*. Although the tetraploid cytotype of *H. pilosella* is generally sexually reproducing and self-incompatible, Gadella (1984) has found some field collections of tetraploid *H. pilosella* that are facultatively apomictic (termed apo-amphimictic by Gadella, loc. cit.). Skaliñska and Kubieñ (1972) reported that the tetraploid *H. pratense* (= *H. caespitosum*) produced predominantly unreduced embryo sacs but in expanded flower heads they also found mature embryo sacs that were apparently meiotic in origin, thus suggesting facultative apomixis. Pentaploid

cytotypes of *H. aurantiacum*, *H. caespitosum*, *H. pilosella* and *H. praealtum* have all been found to be apomictic. All apomictic members of the subgenus *Pilosella* were at one stage described as facultative apomicts (Turesson and Turesson, 1960).

Turesson and Turesson (1960) had interpreted the lack of seed development in some florets of pentaploid *H. pilosella* after emasculation as meaning that those florets were sexual, requiring fertilisation. However, Turesson (1972) later reviewed the evidence. Turesson and Turesson (1960) had found a similar number of pentaploid *H. pilosella* florets not developing seed, regardless of the presence of pollination. Turesson (1972) could see no evidence of the pentaploid *H. pilosella* reproducing sexually. After extensive experimentation, Gadella (1972, 1987) found only evidence of apomictic reproduction in field collections of pentaploid *H. pilosella*, and concluded that it was highly probable that these populations only produced seed by apomixis. Turesson (1972) observed that the sexual embryo sac of the pentaploid shrivelled and died even before the apomictic embryo sac had grown down from the nucellus. Similarly, Skaliñska (1971) reported that the sexual embryo sac of pentaploid *H. aurantiacum* dies at a very early stage after irregular meiosis.

Gadella (1987) obtained tetraploid and pentaploid *H. pilosella* progeny (plus one addition nanoploid, 2n=81) by crossing pollen from pentaploid apomicts (containing two or three full sets of chromosomes) with sexual tetraploid mother plants (sexual embryo sacs containing two sets of chromosomes). Genes for apomixis were not always present in the pollen of the pentaploid apomict. Thus the progeny included apomictic tetraploids and sexual pentaploids. Gadella produced further sexual pentaploid plants from crossing tetraploid and hexaploid sexual parents. Gadella found that progeny of the sexual pentaploid plants, after pollination from sexual tetraploid and hexaploid *H. pilosella*, were often aneuploid but a large proportion were euploid, implying a large proportion of euploid embryo sacs. Ploidy levels of the progeny indicated that these euploid embryo sacs could be diploid or triploid.

Gadella (1991) gave the numbers of chromosomes of progeny from sexual pentaploid *H. pilosella* that had been produced by crossing sexual tetraploids with sexual

hexaploids. When the sexual pentaploid was pollinated by a tetraploid (diploid pollen), 32 progeny were obtained. Four were tetraploid and seven pentaploid, while 21 were aneuploid (2n=38,39,40,42,44). When pollination was from a hexaploid (triploid pollen), 35 progeny were obtained, including 11 pentaploid, eight hexaploid and 16 aneuploid plants (2n=46,48,49,50,51,52). Combining these two crosses, an average of 55% of progeny were aneuploid, presumably due to aneuploid embryo sacs of the pentaploid. It is possible that an even larger proportion of embryo sacs may have been aneuploid but aborted due to aberrant chromosome numbers. If the sexual pentaploid plants can produce a significant proportion of euploid embryo sacs, then it may well be possible for apomictic pentaploid *H. pilosella* to produce euploid sexual embryo sacs. Evidence of sexual reproduction in apomictic pentaploid *H. pilosella* may be lacking simply because of the rareness of the event. The conditions necessary for investigators to have observed sexual progeny through chromosome counting included 1) the sexual embryo sac did not abort and 2) escaped competition with the apomictic embryo sac growing down, 3) was pollinated by compatible pollen, 4) subsequent progeny must have survived for examination, and 5) the progeny's chromosome number must have differed from the mother plant.

Differences in apomictic nature exist between some species of *Hieracium* subgenus *Pilosella*, and have been demonstrated to exist between ploidy levels within species and even within tetraploid *H. pilosella*. Therefore care should be taken in making assumptions on one cytotype based on evidence for another. Generally, however, there is no direct evidence of field collected pentaploids (of any *Hieracium* species) reproducing sexually. If sexual reproduction exists in pentaploid *H. pilosella* it is apparently rarely successful and its detection may require more refined techniques than those used to date. Genetic markers are likely to aid in such investigations. The extent and speed of the invasion of large tracts of New Zealand high country by pentaploid apomictic *H. pilosella* provides much opportunity for sexual reproduction by the pentaploids if it is at all possible.

Throughout the two years spent in study in Europe, large numbers of *H. pilosella* seed collected from the field were propagated. No aneuploids (which may have indicated a

degree of sexual reproduction) were found in chromosome investigations or noticed as particularly weak plants amongst the groups of seedlings. The only variation in ploidy levels found in New Zealand *H. pilosella* was the presence of hexaploids at the Haldon Station locality (HpilHAs). The high proportion of hexaploids among the seedlings examined from this locality indicated that some of the parent plants at that locality were probably already hexaploid and the hexaploids were not likely to have arisen spontaneously from chance sexual events. Five seedlings from all 25 other localities were examined; only pentaploids were recorded for all 125 plants. Assuming that sexual crossing and somatic meiosis would produce tetraploid, pentaploid and hexaploid progeny with equal probability, the chance that a 3% rate of non-apomictic achene production would result in no tetraploids or hexaploids out of 125 plants was less than 5% ($p=0.044$). It should be noted that the five *H. pilosella* plants per locality included in the above calculation were each replicated five times, non- replicated counts were also made for each of the localities again showing no evidence of tetraploid, hexaploid or aneuploid numbers.

Somatic meiosis has not been reported for certain in *Hieracium* but should still be considered. Christoff and Christoff (1948) treated pentaploid apomictic *Hieracium hoppeanum* seed with colchicine to produce a decaploid plant. Some progeny from the decaploid plant was pentaploid which prompted the authors to assume that somatic meiosis had taken place. Turesson and Turesson (1960), however, pointed out that colchicine induced polyploids often included chimeras of the original ploidy level and therefore the pentaploid progeny of Christoff and Christoff's plant was no surprise.

Several of the rust experiments and screenings (Chapter V) used host plants that were grown from achenes of the same capitulum. The probability of an achene being produced sexually or through somatic meiosis was less than 3%, if it occurred at all, meaning that an assumption of genetic similarity between achenes was reasonable for the purposes of rust work. Although obligate apomixis is possible in pentaploid *H. pilosella*, that only refers to achene production and in fact good pollen has been produced by this cytotype.

VII.1.5 IMPLICATIONS OF PLOIDY LEVELS FOR *HIERACIUM* AUTECOLOGY AND CONTROL

The existence of ploidy level variation in *H. aurantiacum*, *H. caespitosum*, *H. pilosella* and *H. praealtum* demonstrates infraspecific genetic variation in New Zealand plants. This should be taken into account when investigating autecology (including breeding system and invasiveness) and potential control measures. The presence of apomixis in New Zealand *H. pilosella*, even if not accounting for all of seed output, implies a reduced capacity for variation. Restricted variation means less variants to consider for control with highly specific control measures, e.g., some fungal biological control agents. A reduced capacity for producing variation also implies reduced ability to evolve genetic non-susceptibility or resistance to a control agent.

The genetic potential for interspecific hybridisation has been discussed and may need to be a consideration for *Hieracium* control if hybrids contain the weed potential of one parent and non-susceptibility or resistance to a control method of the other parent. For instance, *H. caespitosum* has not shown susceptibility to any of the rust isolates investigated (Sections I.5.1.b and III.7). Hybridisation is common amongst all members of the subgenus *Pilosella* in Europe (Gadella, 1992) and between many of the subgenus *Pilosella* taxa naturalised in North America (Lepage, 1967). The extent of hybridisation in Europe and North America is no doubt aided by the presence of tetraploid (sexually reproducing) cytotypes of *H. pilosella* (Vander Kloet, 1978, recorded tetraploid *H. pilosella* in Nova Scotia). Hybrids can be sexually reproducing, apomictic or in some cases sterile, depending partly on the ploidy level and the presence of genes for apomixis. Interspecific hybridisation may have already occurred in New Zealand. Garnock-Jones (in Webb *et al.*, 1988) notes that one New Zealand herbarium specimen was probably *H. pilosella* X *H. praealtum*, while others may have been hybrids between *H. caespitosum* and *H. praealtum*.

VII.1.6 PLOIDY LEVELS OF BRITISH ISLES *HIERACIUM* SPECIES

VII.1.6.a Introduction

The ploidy levels of some *Hieracium* from the British Isles were examined. Some of the plants investigated were the source of rust isolates used in the pathogenicity work and thus the relationship between pathogenicity and ploidy level was tested. The ploidy level results for each species contributed to the general knowledge of *Hieracium* spp. ploidy level distributions.

VII.1.6.b Methods

All plants were transplanted from the field and grown on in glasshouse conditions. The species and localities of their collection are given in Table 7.2. *H. pilosella* was collected from 23 localities, *H. aurantiacum* from two localities, *H.* x *stoloniflorum* from one locality and *H. murorum* of subgenus *Hieracium* from one locality. Counts were made from transplanted rosettes growing in individual pots, filled with 1 part John Innes potting mix No.2: 1 part sphagnum moss peat. Methods for selection of roots and chromosome analysis were as described in Section VII.1.2.

VII.1.6.c Results

The results are presented in Table 7.2. Most British Isles populations sampled were *H. pilosella*. For this species, almost all Scottish collections of the 15 tested proved to be pentaploid except for one population from Kyle of Lochalsh which was heptaploid. Pentaploids were evident in northern England as were tetraploids. A hexaploid was identified from Cornwall and tetraploids from the southern Lake District and north Wales.

Table 7.2. Ploidy levels of British Isles *Hieracium* populations.

Species	Grid Reference	Origin of Plant	Host of Isolate	Ploidy Level
H. pilosella	NH 6633	S of Inverness III, Highlands		5X
H. pilosella	NG 7627	Kyle of Lochalsh, west Scotland		7X
H. pilosella	NH 8425	Slochd I, Highlands		5X
H. pilosella	NH 8425	Slochd III, Highlands		5X
H. pilosella	NH 8912	Aviemore I, Highlands		5X
H. pilosella	NH 8912	Aviemore II, Highlands		5X
H. pilosella	NJ 8909	S of Aviemore I, Highlands	BRIT32	5X
H. pilosella	NH 8909	S of Aviemore III, Highlands		5X
H. pilosella	NN 1472	Ben Nevis I, Highlands	BRIT2	5X
H. pilosella	NN 1472	Ben Nevis II, Highlands	BRIT3	5X
H. pilosella	NH 4469	Sanna Bay, west Scotland	BRIT53	5X
H. pilosella	NN 6740	Ben Lawers, Highlands		5X
H. pilosella	NT 2773	Salisbury Crags I, Edinburgh	BRIT1	5X
H. pilosella	NT 2772	Hillside Arthur's Seat, Edinburgh		5X
H. pilosella	NT 2370	Craiglockhart Hill, Edinburgh		5X
H. pilosella	NT 7405	Catcleugh Reservoir, Cheviot Hills		5X
H. pilosella	NY 7567	Hadrians Wall, Yorkshire		5X
H. pilosella	SD 4679	Arnside I, south Lake District		4X
H. pilosella	SH 3374	Rhosneigr I, north Wales		4X
H. pilosella	NY 7864	Bardon Mill Railway, Yorkshire		4X
H. pilosella	SH 8211	Mid Wales I		4X
H. pilosella	SW 0654	E of St Austell I, Cornwall		6X
H. pilosella	52°16'N 7°06'W	County Kilkenny, Ireland	EIRE14	5X
H. x *stoloniflorum*	NT 2185	Garden Weed, Edinburgh		5X
H. aurantiacum	SH 7856	Betwys-y-Coed II, north Wales		4X
H. aurantiacum	NO 5217	St Andrews, East Scotland		3X
H. murorum	NY 7864	Bardon Mill Railway, Yorkshire		3X

Two populations of *H. aurantiacum* were sampled, one tetraploid from north Wales and one triploid from the East Coast of Scotland. *H. murorum* (subgenus *Hieracium*) from Yorkshire was found to be triploid.

The original hosts of three of the more infective British isolates (judged on experiments in Section V.3.1, V.3.2 and V.3.4) were investigated for ploidy level and all were pentaploid. The plants were from Sanna Bay, Slochd I and S of Aviemore I and were host to isolates BRIT53, BRIT 21 and BRIT32 respectively. The most pathogenic isolates of rust on New Zealand *H. pilosella* came from Ireland (Section V.3) and the host of isolate ÉIRE14 was investigated and also found to be pentaploid.

VII.1.6.d Discussion

The results of these limited chromosome number investigations carried out on plants collected from throughout the British Isles are largely in agreement with the literature. Bishop and Davy (1994) summarise the knowledge of ploidy levels of *H. pilosella* present in Britain. Tetraploid cytotypes are most common in the South and pentaploid cytotypes are predominant in Scotland. Gadella (1972) found only pentaploid *H. pilosella* when he examined five samples from the Scottish Highlands and one from the east coast. Hexaploids are known to be present in Britain (Bishop and Davy, 1994) and diploids and triploids have been reported from south-east England (H. Misirdali, in Bishop and Davy, 1994). The present work found a heptaploid representative of *Hieracium pilosella*, a first record for Britain, at Kyle of Lochalsh, Scotland.

The apparent link between high ranking in infectivity screening on New Zealand *H. pilosella* and the chromosome count of pentaploid on source plants of the isolates is confounded by several factors. For instance, the vast majority of isolates were from Scotland and most were likely to have been pentaploid, hence the probability of finding the most infective isolates on pentaploid hosts was higher. Gadella (1972) reported tetraploid (12 samples), pentaploid (6 sample) and hexaploid (1 sample) from throughout Ireland, indicating that pentaploid *H. pilosella* constituted a significant proportion of the cytotypes present but was less common than the tetraploid cytotype.

VII.2 ISOZYME ELECTROPHORESIS

VII.2.1 INTRODUCTION

There are visible differences in the morphology of *H. pilosella* throughout New Zealand but the extent to which this is phenotypic plasticity rather than genetically controlled is uncertain. Isozyme electrophoresis was employed to investigate whether genetic variation exists within New Zealand pentaploid *H. pilosella*.

Isozyme (or allozyme) electrophoresis has been employed in a number of other weed biological control programmes. Sometimes the aim has been to clarify the interspecific variation present, e.g., between *Prosopis* L. taxa (Panetta and Carstairs, 1989). In other programmes the aim was to identify infraspecific variation, e.g., within Australian *Chondrilla juncea* (Burdon *et al.*, 1980) and within *Xanthium strumarium* (Moran and Marshall, 1978).

Isozyme electrophoresis involves the analysis of enzyme forms present in an organism. Variation in isozymes between individuals implies a genetic difference. Buffered extractions of proteins from live tissue are placed on a buffered starch gel; an electric current is applied through electrode buffers, causing migration of the proteins. The enzyme systems of interest are then selectively stained. Different forms of the same enzyme (isozymes) will migrate at differing rates and thus the migration distance after a period can be used to compare isozymes. With good resolution, a band or bands of isozymes are evident.

Some of the factors that can affect resolution are the quality of the tissue and the recipes of the extraction buffer, gel buffer, electrode buffer and the stain. Much of the work undertaken in the present study aimed to optimise recipes of buffers and stain for each enzyme system of *H. pilosella*.

The objective of this isozyme electrophoresis study was to establish knowledge of the variation present and allow the selection of differing genotypes to include in rust pathogenicity experiments. Isozyme electrophoresis also served the purpose of

identifying enzyme systems that could be used in future taxonomic studies of *H. pilosella.*

Work was undertaken in three institutes. A pilot study at the School of Forestry, University of Canterbury was followed up by intensive work at St Andrews University, UK and the CSIRO Biological Control Unit in Montpellier, France.

VII.2.2 ENZYME SYSTEM CODES

In this section, the standard enzyme code is given for each enzyme system studied and the international enzyme number is also given (Table 7.3).

Table 7.3. Enzyme system codes.

Enzyme System	Code	Enzyme Number
Aconitase	ACO	E.C. 4.2.1.3
Acid phosphatase	ACP	E.C. 3.1.3.2
Alcohol dehydrogenase	ADH	E.C. 1.1.1.1
Fructose bisphosphate aldolase	FBA	E.C. 4.1.2.13
Diaphorase	DIA	E.C. 1.6.4.3
Esterase (alpha)	ESTα	E.C. 3.1.1.-
Esterase (beta)	ESTβ	E.C. 3.1.1.-
Fumarase	FUM	E.C. 4.2.1.2
Glutamate dehydrogenase	GDH	E.C. 1.4.1.2
Glucose oxidase	GO	
Glutamic-oxalaoacetate transaminase	GOT	E.C. 2.6.1.1
Glucose-6-P-dehydrogenase	G-6-PDH	E.C. 1.1.1.49
Hexokinase	HEX	E.C. 2.7.1.1
Isocitrate dehydrogenase	IDH	E.C. 1.1.1.42
Leucine-amino peptidase	LAP	E.C. 3.4.11.1
Lactate dehydrogenase	LDH	E.C. 1.1.1.27
Malate dehydrogenase	MDH	E.C. 1.1.1.37
Menadione reductase	MNR	
Malic Enzyme	ME	E.C. 1.1.1.40
Peroxidase	PRX	E.C. 1.11.1.7
6-Phosphogluconate dehydrogenase	PGD	E.C. 1.1.1.44
Phosphoglucoisomerase	PGI	E.C. 3.2.1.23
Phosphoglucomutase	PGM	E.C. 5.4.2.2
Shikimic dehydrogenase	SKD	E.C. 1.1.1.25
Superoxide dismutase	SOD	E.C. 1.15.1.1
Succinate dehydrogenase	SuDH	
Triose phosphate isomerase	TPI	E.C. 5.3.1.1

VII.2.3 PILOT STUDY

VII.2.3.a Introduction

A pilot study was initiated to assess the potential of isozyme electrophoresis in identifying variation in New Zealand *Hieracium*.

VII.2.3.b Materials and methods

Work was based at the School of Forestry, Canterbury University. Nine enzyme systems were investigated, these were EST(α + β), ACP, MNR, PGI, ACO, SKD, MDH, GDH, PGM. Most enzymes were studied with the methods of Haase (1992). The enzyme systems of ACP, GDH and MNR were analysed on the ridgeway buffer system according to the methods in Billington (1989). Single plants of pentaploid *H. pilosella* ssp. *micradenium* were sampled from Geraldine (HpilGEr), Cannington (HpilCAn), Cave (HpilCAv), Craigmore Valley (HpilCVa) and Maryburn Station (HpilMAs) and Hinewai Reserve (HpilHBp). Small portions of the youngest leaves were taken from rosettes that had been transplanted from the field and grown in glasshouse conditions.

VII.2.3.c Results

The *H. pilosella* samples showed activity for all the enzyme systems tested (Table 7.4). No difference was seen between samples, although resolution was poor for ACP and there was no interpretable result for EST (α and β together).

Table 7.4. Results of isozyme electrophoresis analysis at University of Canterbury, (including the quality of resolution achieved whether a difference between could be seen between individual *H. pilosella* samples).

ENZYME SYSTEM	RESOLUTION	RESULT
EST(α + β)	poor	-
ACP	poor	appeared similar
MNR	poor-satisfactory	no diff.
PGI	satisfactory	no diff.
ACO	satisfactory	no diff.
SKD	satisfactory	no diff.
MDH	satisfactory	no diff.
GDH	satisfactory	no diff.
PGM	good	no diff.

VII.2.3.d Discussion

Despite the geographical separation of the sample sites, there were no visible differences in the seven resolved enzyme systems. The resolution of these enzymes was sufficient to warrant further investigation of the variation present in New Zealand *Hieracium*.

VII.2.4 ST ANDREWS UNIVERSITY: Optimising Enzyme Extraction And Stain Buffer pH

VII.2.4.a Introduction

The second round of isozyme electrophoresis involved a larger number of enzyme systems. The aim was to identify variation and optimise resolution of banding of each of the enzyme systems by modifying the amount of extraction buffer and the pH of the stain buffer.

VII.2.4.b Materials and methods

Work was based at St Andrews University, United Kingdom. Twenty three enzyme systems were tested for variation between New Zealand *Hieracium* taxa and between

ten *H. pilosella* collections. The enzyme systems tested are listed in Table 7.5. The methods employed were modified from those of Ashton (1990), developed for another herbaceous Asteraceae genus, *Senecio*. Modifications were made to the concentration of extract from each plant sample; either one or two drops of extraction buffer to the same amount of leaf tissue. Modifications were also made to identify the optimum pH of the enzyme stain buffers; pH levels of 7.5, 8.0 and 8.5 were tested for each of the enzymes except ACP, and EST (α and β).

VII.2.4.c Results

Reasonable resolution was achieved in eight enzyme systems. Other systems were either inconsistent for the samples tested, poorly resolved, or lacked visible activity. The results for each enzyme system are given in Table 7.5. The stain buffer pH levels usually had a large effect on the resolution of banding for the enzymes and the optimum stain buffer pH level is given for several enzyme systems. For most enzyme systems the optimum pH level was that used for *Senecio* by Ashton (1990). Results were improved with changes in pH levels for the enzymes GDH, IDH, ME and PGM. Whenever activity was discernible, the activity was best when extraction was with one rather than two drops of buffer.

VII.2.4.d Discussion

Eight enzyme systems displayed good potential for identifying variation within *H. pilosella*. These were ACO, GDH, GOT, IDH, ME, PGD, PGI and PGM. The variants identified with these enzyme systems are presented in Section VII.2.6.c. The improved results with one rather than two drops of extraction buffer were because one drop of extraction buffer would have resulted in a higher concentration of enzymes than two drops. One of the main functions of the extraction buffer is to reduce the effect of plant phenolics which interfere with enzyme activity. Therefore it appears that the level of plant phenolic action in the *Hieracium* samples did not warrant extra extraction buffer.

Table 7.5. Resolution quality and optimum stain buffer pH for isozyme electrophoresis on *Hieracium*.

Enzyme System	Optimum pH (stain buffer)	Resolution
ACO	8.0	satisfactory
ACP	(not tested)	satisfactory
ADH	8.0	some activity to no activity
ESTα	(not tested)	poor
ESTβ	(not tested)	poor
FBA	?	no activity
FUM	?	no activity
GDH	7.5	excellent
GOT	8.5	excellent
G-6-PDH	8.0	poor
HEX	?	poor
IDH	8.0	satisfactory
MDH	8.5	poor
MNR	?	no activity
ME	7.5	satisfactory
PRX	?	no activity
PGD	8.0	satisfactory
PGI	8.5	satisfactory
PGM	8.0	satisfactory
SKD	?	very faint to no activity
SOD	?	poor
SuDH	?	no activity
TPI	?	no activity

VII.2.5 MONTPELLIER: Optimising Gel And Electrode Buffer Systems

VII.2.5.a Introduction

The gel and electrode buffers used can significantly affect the activity and resolution of enzymes. The aims of this study were to identify variants of New Zealand *H. pilosella* and identify the optimum gel and electrode buffer systems for several enzymes.

VII.2.5.b Materials and Method

Work was based at the CSIRO Unit of Biological Control, Montpellier, France. Seventeen enzyme systems, most of which were investigated in the St Andrews University study (Section VII.2.4), were examined to identify optimum gel and electrode buffer systems. The buffer system recipes tried are listed in Table 7.6. Seven samples of New Zealand *H. pilosella* were included in the testing of 12 enzyme systems (detailed in Table 7.7). The methods used were small modifications of the CSIRO, Montpellier procedures (C. Espiau pers. comm.). Stain recipes for the 12 enzymes were taken from St Andrews University (Ashton, 1990). The resolution of the 12 enzymes was examined on two buffer systems, tris-citrate and Ridgeway, with recipes taken from Montpellier CSIRO.

Enzymes not showing significant activity in the above buffer systems were then tested on the tris-citrate buffer system according to the recipes of St Andrews University. Four further enzymes, diaphorase, glutamate dehydrogenase, leucine-amino peptidase and lactate dehydrogenase, were tested for activity on a histidine buffer system.

Table 7.6. Buffer System recipes. (As modified by Montpellier CSIRO, St Andrews University and Forestry School, University of Canterbury from standard recipes).

Buffer System	Electrode Buffer	Gel Buffer
Ridgeway (Montpellier)	Tris 3.6 g Citric Acid 1.05 g Distilled Water 1000 ml (adjusted to pH 8.5)	Lithium Hydroxide 2.52 g Boric Acid 18.55 g Distilled Water 1000 ml (adjusted to pH 8.1)
Tris-citrate (Montpellier)	Tris 16.35 g Citric Acid 9.04 g Distilled Water 1000 ml (adjusted to pH 7.0)	Tris-citrate electrode buffer 50 ml Distilled Water 950 ml (pH 7.0)
Tris-citrate (St Andrews)	Tris 16.35 g Citric Acid 6.1 g Distilled Water 1000 ml (adjusted to pH 8.0)	Tris-citrate electrode buffer 67 ml Distilled Water 950 ml (pH 8.0)
Histidine (Canterbury)	Tris 15.135 g 1M Citric Acid 25 ml Distilled Water 1000 ml (adjusted to pH 7.0)	L-Histidine 2.1 g EDTA 0.08 g 1.0M Tris 8 ml Distilled Water 1000ml

VII.2.5.c Results

Good to excellent resolution was achieved with eight enzymes. Activity on both of the tris-citrate systems was poor for most enzymes. Two enzymes that were not tested on *Hieracium* previously, DIA and LAP demonstrated excellent resolution.

Table 7.7. Resolution quality and the optimum buffer systems for 12 enzyme systems.

Enzyme System	Preferred Buffer System	Resolution
ACO	?	very faint
ACP	?	poor
ESTα	?	poor
ESTβ	?	poor
GOT	Ridgeway	excellent
HEX	?	very faint
IDH	Ridgeway	good
MDH	Ridgeway	very faint
ME	Ridgeway	excellent
PGD	Ridgeway	good
PGI	Ridgeway	poor
PGM	Ridgeway	excellent
DIA	Histidine[a]	excellent
FUM	?	no activity
GDH	Ridgeway[b]	excellent
LAP	Histidine[a]	excellent
LDH	?	little activity

[a] Although DIA and LAP were not tested on other buffer systems the Histidine system provided excellent resolution.
[b] GDH resolution was excellent on Histidine but resolution was better in St Andrews with the Ridgeway system.

VII.2.5.d Discussion

The reason for the tris-citrate buffer systems not providing good activity from most enzymes was uncertain. Some of the same enzymes had had good activity on tris-citrate in St Andrews. Between the other two buffer systems there were eight enzymes displaying sufficient activity and resolution for analysis of the variation between samples.

The results indicated that the best nine enzymes for assessing the variation in *H. pilosella* based on their resolution were, DIA, GDH, GOT, IDH, LAP, ME, PGD, PGI and PGM. The variants identified with these enzyme systems are detailed in Section VII.2.6.c.

VII.2.6 ISOZYME VARIATION PRESENT IN NEW ZEALAND *HIERACIUM*

VII.2.6.a Introduction

The pilot study conducted at University of Canterbury did not indicate variation between samples in the enzyme systems. The electrophoresis work at St Andrews and Montpellier, however, involved a wider range of plants and a larger number of enzymes. Variation was found to be present within New Zealand *H. pilosella.*

VII.2.6.b Materials and methods

Isozyme electrophoresis was employed on 24 rosettes grown from New Zealand seed collected at various locations. Nine enzyme systems including two loci of DIA were studied on varying numbers of the plants. Ploidy levels had been ascertained for each plant. A further three collections were of New Zealand *H. aurantiacum*, *H. praealtum* and *H.* x *stoloniflorum* but these were only included to check for differences between species and not replicated or analysed. All rosettes were kept in long-day glasshouse conditions with supplementary artificial light.

At the University of St Andrews, ten samples of New Zealand *H. pilosella* from six localities (Table 7.8) were studied for four enzyme systems, GDH, PGM, GOT and IDH.

At CSIRO Montpellier, seven samples of New Zealand *H. pilosella* from five localities (Table 7.8) were tested for nine enzymes (Table 7.10). All plants sampled were grown from seed and kept in glasshouse conditions. The enzyme systems tested on a lithium borate buffer system were GOT (glutamate oxaloacetate transaminase), ME, 6-PGD (6-phosphogluconate dehydrogenase), PGI (phosphoglucose isomerase), and PGM (phosphoglucomutase). The stain recipes for these enzymes were from

Ashton (1990) with modified buffer pH's according to the optimum results in the St Andrews work. The enzyme systems tested with a histidine buffer system were DIA (diaphorase, 2 loci), GDH (glutamic acid dehydrogenase), and LAP (leucine-amino peptidase). Stain recipes were unmodified from the recipes of the CSIRO, Montpellier. A sample of seven further plants was later investigated for patterns of the LAP enzyme.

Table 7.8. *H. pilosella* samples analysed by isozyme electrophoresis at St Andrews and Montpellier.

CODE	Location	Grid Reference	Ploidy Level
	St Andrews University		
HpilHA(i)	Haldon Station	44°21.0'S 170°17.0'E	5X
HpilHOs(i)	Holbrook Station	44°05.2'S 170°31.9'E	5X
HpilHOs(ii)	Holbrook Station	44°05.2'S 170°31.9'E	5X
HpilLCo(i)	Lake Coleridge	43°20.4'S 171°37.4'E	5X
HpilLCo(ii)	Lake Coleridge	43°20.4'S 171°37.4'E	5X
HpilLRu(i)	Lake Ruataniwha	44°15.9'S 170°04.4'E	5X
HpilLRu(ii)	Lake Ruataniwha	44°15.9'S 170°04.4'E	5X
HpilMAs1	Maryburn Station (I)	44°11.1'S 170°27.7'E	5X
HpilMJt(i)	Mt John trial site	43°59.1'S 170°27.7'E	5X
HpilMJt(ii)	Mt John trial site	43°59.1'S 170°27.7'E	5X
	Montpellier CSIRO		
HpilHAs(ii)	Haldon Station	44°21.0'S 170°17.0'E	5X
HpilHAs(iii	Haldon Station	44°21.0'S 170°17.0'E	5X
HpilHAs6X	Haldon Station	44°21.0'S 170°17.0'E	6X
HpilHSp	Hanmer Springs	42°27.4'S 172°54.4'E	5X
HpilLLy	Lake Lyndon	43°17.8'S 171°42.5'E	5X
HpilLRd	Lyndon/Coleridge Rd	43°20.5'S 171°38.2'E	5X
HpilTHs	Tara Hill Station	44°32.2'S 169°54.3'E	5X
HpilSCs2	Stony Creek Station (II)	44°21.3'S 170°23.9'E	5X
HpilHEr	Hermitage (Mt. Cook)	43°44.0'S 170°06.6'E	5X
HpilLCl(i)	Lake Clearwater	43°37.2'S 171°03.6'E	5X
HpilJPa(i)	Jollies Pass	42°27.4'S 172°54.5'E	5X
HpilJPa(ii)	Jollies Pass	42°27.4'S 172°54.5'E	5X
HpilJPa(iii)	Jollies Pass	42°27.4'S 172°54.5'E	5X
HpilLCl(ii)	Lake Clearwater	43°37.2'S 171°03.6'E	5X

VII.2.6.c Results

Variation within New Zealand pentaploid *H. pilosella* were demonstrated with GOT (Fig 7.7), PGI, PGM (Fig 7.9) and possibly IDH, LAP and PGI. The hexaploid *H. pilosella* was distinguishable from the pentaploids with PGD. Differences were seen between *H. aurantiacum, H. praealtum* and *H. pilosella* under GOT, IDH and PGD at least (Table 7.9).

Enzyme resolution at St Andrews University was generally good. GDH (Fig. 7.6) showed no variation even between species of *Hieracium* whereas the other enzyme systems demonstrated variation within the *H. pilosella* samples (Table 7.10). Most other enzymes appeared to show variation within New Zealand *H. pilosella* but this was unreliable.

At Montpellier, resolution was generally very good. Again no variation was apparent with GDH. The remaining enzymes demonstrated differences between *H. pilosella* samples but these were only replicated for GOT, PGD and PGM.

PGM demonstrated clear differences between the two pentaploid HpilHAs samples. The HpilHAs hexaploid matched one of the HpilHAs pentaploids and the samples of HpilTHs and HpilHAs. The HpilLLy and the HpilLRd samples matched each other. LAP (Fig 7.10) demonstrated at least four distinct patterns in the first six New Zealand pentaploid *H. pilosella* samples examined at Montpellier, including variation between two samples from Haldon Station.

The results for IDH, LAP, ME (Fig 7.8) and PGI were only clearly resolved once and although some variation between samples was in evidence for these systems, further replication would be necessary for confirmation.

Table 7.9. Observed value of enzyme systems for identifying variation between *Hieracium* species and within *H. pilosella*.

ENZYME	RESOLUTION	RESULT (differences seen)	
		between species[a]	within NZ *H. pilosella*[a]
DIA	excellent	?	?
GDH	excellent	no	no
GOT	excellent	yes	yes
IDH	good	yes	yes
LAP	excellent	?	yes
ME	excellent	?	yes
PGD	good	yes	yes
PGI	satisfactory	?	?
PGM	excellent	?	yes

? = Probable variation seen.

Table 7.10. Variation in isozyme patterns within New Zealand *H. pilosella*. Different letters indicate a variation in isozyme pattern.

		Enzyme System									
Laboratory	**Host Source**	**DIAi**	**DIAii**	**GDH**	**GOT**	***IDH***	***LAP***	***ME***	**PGD**	***PGI***	**PGM**
St Andrews	HpilHAs(i)			A	A	*A*					A
	HpilHOs(i)			A	A	*A*					A
	HpilHOs(ii)			A	B	*A*					B
	HpilLCo(i)			A	A	*A*					A
	HpilLCo(ii)			A	A	*A*					A
	HpilLRu(i)			A	A	*A*					A
	HpilLRu(ii)			A	A	*B*					A
	HpilMAs1(i)			A	B	*A*					B
	HpilMJt(i)			A	A	*B*					A
	HpilMJt(ii)			A	A	*A*					A
Montpellier	HpilHAs5X(ii)	A	A	A	B		*B*	*B*	B	*B*	B
	HpilHAs5X(iii)	A	A	A	A		*C*	*C*	B	*B*	A
	HpilHAs6X(i)	A	A	A	A		*A*	*A*	A	*A*	A
	HpilHSp(i)	A	A	A	A		*C*	*C*	B	*B*	A
	HpilLLy(i)	A	A	A	A		*E*	*C*	B	*A*	C
	HpilLRd(i)	A	A	A	A		*F*	*B*	B	*A*	C
	HpilTHs(i)	A	A	A	A		*D*	*C*	B	*A*	A
	HpilHEr(i)						*F*				
	HpilJPa(i)						*B*				
	HpilJPa(ii)						*B*				
	HpilJPa(iii)						*B*				
	HpilLCl(i)						*F*				
	HpilLCl(ii)						*E*				
	HpilSCs2(i)						*B*				

NOTE: Italics (for IDH, LAP, ME, PGI) indicate results without replication. These enzyme systems results should only be treated as possible differences.

VII.2.7 DISCUSSION OF ISOZYME ELECTROPHORESIS

The results have demonstrated within and between population variation in New Zealand pentaploid *H. pilosella*. GOT and PGD banding patterns were particularly useful for identifying variant. At both laboratories, a consistency was noted between GOT results and PGM results such that the two patterns noted for GOT (A and B) and the three patterns for PGM (A, B and C) separated the 17 samples tested into three distinct groups; most New Zealand *H. pilosella* samples belonged to the group with five bands of GOT and two strong bands of 6-PGD. The hexaploid *H. pilosella* from Haldon was shown to be distinguishable on the basis of isozyme electrophoresis. *H. aurantiacum* and *H. praealtum* were shown to be easily distinguished from *H. pilosella* under GDH, IDH and PGD.

Richards (1986) listed factors which can result in non-genetic variation isozyme electrophoresis banding patterns. These factors were

- Technical artefacts such as pH and temperature.
- Phenotypic effects, aging etc.
- Dissociation of polymeric enzymes, resulting in multiple bands; and other non-Mendelian segregation.
- Differential fitness of isozyme morphs.
- Non-random mating and other abnormalities of a sexual breeding system such as selfing etc.

Growing plants in similar conditions, taking similar aged leaf tissue and running samples on the same gel minimised the chances of many of the above factors. Replication of some of the enzyme systems demonstrated the reliability of those systems' results. Because of the apomictic form of reproduction, it was not possible to test progeny for non-Mendelian segregation.

Interpretation of genetic variation within New Zealand *H. pilosella* is simple if obligate apomixis can be assumed. If no sexual reproduction has occurred in New Zealand, then the variation is due to multiple introductions of the species (probable), and (or) has arisen from mutation or somatic meiosis. Richards (1986) discusses the possible significance of mutation and somatic meiosis in providing variation in agamic complexes of the related genus, *Taraxacum*. The reliability of an assumption of no sexual reproduction has been discussed (Section VII.1.4). Given the large scale invasion of *H. pilosella* in New Zealand there would have been great potential for sexual reproduction if it was at all possible for the pentaploid *H. pilosella*. Hexaploids can have a degree of, or even complete, sexual reproduction, and the discovery of this ploidy level at the Haldon Station locality (HpilHAs) further questions an assumption of no sexual reproduction.

A large degree of variation was indicated by the LAP system, although due to lack of replication, the result was not definite. Confirmation of genetic variation would also have involved checking progeny but that was beyond the scope of this project, which aimed to identify probable variants to be included in rust pathogenicity work.

The biological control programme for apomictic *Chondrilla juncea* (skeleton weed) also involved isozyme electrophoresis work. Three forms of *C. juncea* had been distinguished in Australia on the basis of leaf shape and several characters including inflorescence morphology (Hull and Groves, 1973). There was sometimes difficulty in identifying *C. juncea* forms in the field because of plasticity of characters and one form possessed intermediate characters between the other two forms. Burdon *et al.* (1980) used isozyme electrophoresis to demonstrate differences between the three recognised morphological forms ('A', 'B' and 'C') of the species in Australia. There was an apparent lack of variation within each form. Activity was seen in thirteen of the fourteen enzyme systems employed. No differences were seen between the forms in five (38%) of the thirteen enzyme systems. GDH was found to show differences between form 'B' and the other two. LAP demonstrated differences between all three forms and GOT distinguished form 'C' from the other two. The other enzyme systems to show variation were ADH, EST, MNR, PGM and ACP. Eight samples

were analysed for each of the three plant forms, but the aim of the work was to investigate differences between and not necessarily within the forms.

Isozyme electrophoresis was also employed to investigate variation within the biological control candidate *Xanthium strumarium* L. Moran and Marshall (1978) found variation between known races and very little variation within races based on 13 enzyme loci. *X. strumarium* was described by Moran and Marshall (1978) as a predominantly self pollinated, monoecious, annual.

Isozyme electrophoresis work demonstrated variation within the apomictic taxon *Taraxacum officinale* naturalised in the USA (Lyman and Ellstrand, 1984). The investigators found 21 different isozyme phenotypes after analysing 518 individuals of the species with three enzyme systems (ADH, PGI and PGM). The isozyme variation combined with several morphological characters could discern 47 variants in total. Lyman and Ellstrand (1984) suggested that the numerous variants identified probably resulted from multiple introductions from the taxon's native range of Europe. Interpretation of these results requires caution due to the taxonomic complexity of *Taraxacum* and particularly that *T. officinale* is in fact a large species complex. Ford and Richards (1985) worked with a number of *Taraxacum* agamospecies and found isozyme variation within and between them.

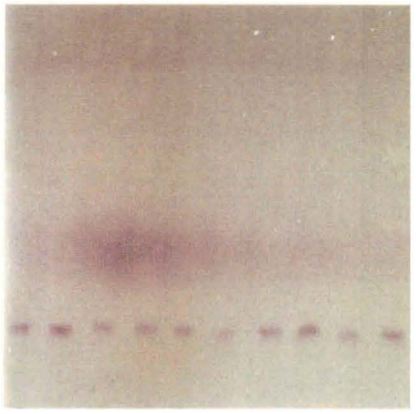
The preceding examples of variation in naturalised apomictic species, *C. juncea* and *T. officinale*, bear similarities to the scenario of New Zealand *H. pilosella*. All three species have shown isozyme variation in their new environment. One of the sources of this variation is likely to be multiple introductions of each of the species but the possibility remains that a degree of sexual reproduction, somatic meiosis, or mutation are responsible for variation.

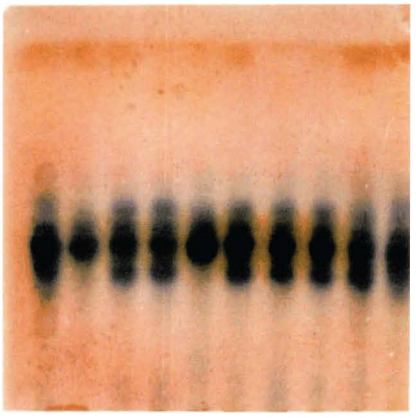
Fig 7.6 (left) **Isozyme electrophoresis GDH**
ENZYME GDH (Glutamate Dehydrogenase).
GDH pattern A (Rf value 0.23)

Fig 7.7 (right) **Isozyme electrophoresis GOT**
ENZYME GOT (Glutamic-oxaloacetate Transaminase).
GOT pattern in most lanes and pattern B in lanes (from left) 2 and 5. (Pattern B lacks lower and upper bands at Rf 0.37 and 0.55 respectively).

Fig 7.8 (left) **Isozyme electrophoresis ME**
ENZYME ME (Malic Enzyme)
ME patterns A (lane 7), B (lanes 1 and 6), and C (lanes 2,3, 4, and 5).

Fig 7.9 (right) **Isozyme electrophoresis PGM**
ENZYME PGM (Phosphoglucomutase)
PGM patterns A (lanes 2,4,5, and 7), B (lane 6), and C (lanes 1 and 3).





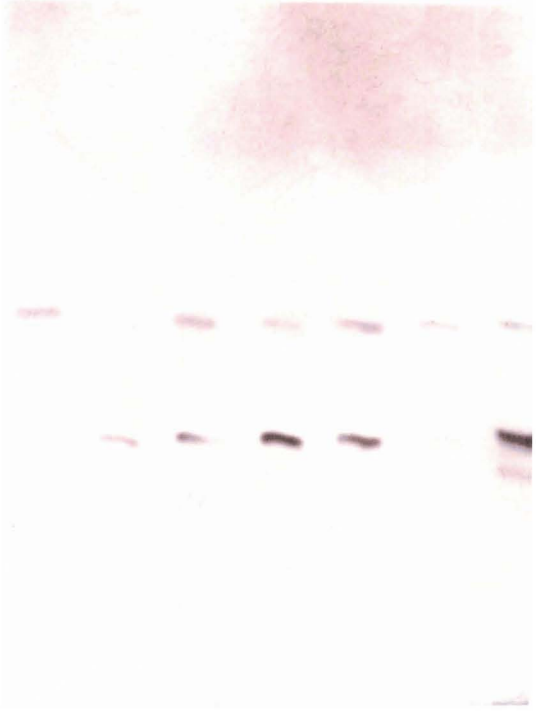


Fig 7.10 **Isozyme electrophoresis LAP.**
ENZYME LAP (Leucine-amino peptidase)
LAP patterns A (lane 7 from left), B (lane 5), C (lanes 3 and 6), D (lanes 1 and 4) and E (lane 2).

The isozyme electrophoresis results for New Zealand *Hieracium* spp. were utilised directly in the rust pathogenicity work. Achenes were collected from the same capitula that produced the achenes sown for the isozyme work. These achenes were used to provide rosettes for several of the experiments in Sections III and V. Investigations could therefore be made into the differential susceptibility, to rust isolates, of the host variants identified.

VII.3 IMPLICATIONS OF HOST TAXONOMY RESEARCH

The ploidy levels of *Hieracium* taxa and their ecological significance have been the subject of much investigation in Europe (Section I.3.6). The present study has shown the value of chromosome counting in assessing the variation of taxa present in New Zealand. The interspecific taxonomy of some *Hieracium* taxa has been chemotaxonomically investigated, through the use of phenolics (Bate-Smith *et al.*, 1968) and flavenoids (Guppy and Bohm, 1976). The present thesis has demonstrated the usefulness of isozyme electrophoresis to detect infraspecific variation in *H. pilosella.*

Chromosome analysis and isozyme electrophoresis demonstrated genetic variation within several *Hieracium* species in New Zealand, particularly *H. pilosella.* In addition, morphological data suggests that at least three taxa (subspecies or varieties) of *H. pilosella* are present in New Zealand (Section 1.3.1.e). The development of fungal biological control should take these variants into account. Several rust pathogenicity experiments (Chapters III and V) included the identified variants of *H. pilosella.*

When a species invades a new country it goes through a "founder effect" such that a limited amount of genetic variation is present in the introduced individuals that manage to naturalise. Nei *et al.* (1975) described this as a "bottleneck effect", the variation present being only a subset of that in the native range. Each of the *Hieracium* species introduced to New Zealand would have gone through this

bottleneck. The presence of genetic variation between individuals is a sum of the variation initially introduced and subsequent variation produced.

The lack of evidence for any sexual activity by the predominant cytotype of *H. pilosella* in New Zealand implies almost obligate apomixis. There are still various avenues to produce a small proportion of genetic variation (Section VII.2.7). The variation present would have arisen from multiple introductions of *H. pilosella*, mutation and/or somatic meiosis. If sexual reproduction exists in pentaploid *H. pilosella*, it must be at a low level; this could be investigated further.

Genetic variation in an invading species includes variation present between individuals and within individuals. Between individual variation is reduced in apomictic and vegetatively reproducing *Hieracium* taxa. The genetic variation within individuals is however potentially large due to the high ploidy level (and thus the large pool of genes available) of most of the taxa (see Section I.3.6). This could explain the adaptability of *H. pilosella* to different niches in the New Zealand high country. Ford (1981) demonstrated that different agamospecies of apomictic *Taraxacum* had differential adaptation to cohabitation with grass species. Differential adaptations have not yet been demonstrated within New Zealand *H. pilosella*.

The ploidy levels and morphological forms of *H. pilosella* in New Zealand represent a small subset of those described in Europe.

Chapter VIII: GENERAL DISCUSSION

Context of Thesis

Hieracium species are a weed problem over large areas of the South Island high country. The lack of suitable *Hieracium* control measures for many of the affected areas has led to investigation of the potential for biological control. The exploratory work of Scott (pers. comm. 1995a) concentrated on two fungal pathogens, rust and powdery mildew. Both of these pathogens exhibited limitations for use as biological control agents, indicating that more research was necessary before introduction. The rust isolates used exhibited low pathogenicity and a propensity to infect only a proportion of plants inoculated, and although powdery mildew was more pathogenic than rust, it also infected one New Zealand endemic species.

The variation present in New Zealand *Hieracium* had been investigated to some extent. The species and subspecies present in New Zealand were recorded by Garnock-Jones (in Webb *et al.*, 1988) and some work had been undertaken on the ploidy levels of *H. pilosella* and the morphological variation present (Makepeace, 1981). The extent of variation present within a species is potentially significant for a highly specific fungal pathogen and therefore infraspecific variation of *H. pilosella* was researched further in this study.

Potential of Biological Control

The use of classical biological control is suited to weed problems covering extensive areas because of the agent's natural spread and long term presence (Section I.2.2.a). Important aspects to consider when assessing the potential of a biological control agent are the significance of the agent's effect on the target weed and any conflicts of interest associated with control. Host specificity is one of the greatest concerns when an agent is proposed for introduction (Section I.2.1). Other conflicts of interest might include the economic and environmental value of the weed itself.

Biology of Host / Pathogen Interactions

Success of a biological control agent depends on several aspects, namely the susceptibility of the targeted taxon, the interactions with environmental conditions present in weed infested areas, the transmissibility of the agent, and the actual effect of the agent on the target weed.

Results have demonstrated that representatives from all 25 *H. pilosella* populations tested are susceptible to one or more of the selected rust isolates. Within *H. pilosella* there was some variation in the susceptibility to rust isolates between host collections. Comparisons of rust collections demonstrated differences in infectivity between isolates on New Zealand *H. pilosella* plants and detached leaves.

The rust *P. hieracii* var. *piloselloidarum* was found to be able to infect siblings of all the identified genotypes of *H. pilosella* (Section III.5.4). *H. pilosella* is the main target for control but results show that rust isolates selected for that species would also infect *H. praealtum* and *H.* x *stoloniflorum* (Section III.7).

Several isolates, all from the south of Ireland, demonstrated the highest infectivity (Sections V.3.6, V.3.7 and V.3.9). One of these isolates (ÉIRE14) was able to infect all identified genotypes of New Zealand *H. pilosella* tested. The isolate also infected *H.* x *stoloniflorum* and *H. praealtum*. Isolate ÉIRE14 was included in limited host specificity tests and did not infect any non-subgenus *Pilosella* species.

Experiments comparing the effect of rust isolates on variants of New Zealand *H. pilosella* identified by chromosome counting and isozyme electrophoresis showed no significant difference in the effect due to host identity, but groups of *H. pilosella* were often found to have varying susceptibility according to their source locality. Rarely were 100% of inoculated plants infected. The least susceptible population was from Godley Peaks Station (South Canterbury) but after further tests, a proportion of plants from this area were shown to be susceptible to infection by some of the isolates tested (Section III.6).

Non-susceptibility of *H. pilosella* rosettes was attributed to host condition. Some of this effect may have been genetic (e.g., specific resistance genes) but physiological condition of the plant was evidently a significant factor. The observation that some rosettes were infected after a second inoculation after not having displayed infection from a previous inoculation with the same isolate was one form of evidence for non-genetic factors affecting susceptibility (e.g., Section III.5.4). Differential susceptibilities of *H. pilosella* rosettes grown from seed of the same capitulum (and therefore likely to be isogenic) provided further evidence (e.g., Section III.9.1).

Glasshouse experiments demonstrated the transmissibility of *Hieracium* rust over a short distance on New Zealand *H. pilosella* (Section III.4). In the field, high proportions of rust infected *H. pilosella* rosettes within a patch were often observed. *Hieracium* rust was found throughout the European and British range of *H. pilosella* surveyed (Section II.1), and on many isolated patches, indicating that long distance dispersal of the rust is possible.

The thesis has established an understanding of the biology of *Hieracium* rust. Field observations have shown that in some parts of Europe, more than ten percent of populations may be infected with the rust (Section II.1). In some locations a majority of patches were infected, for example the Scottish sites, Aviemore, Pitlochry and Arthur's Seat. Indications were that rust on *H. pilosella* was of the endemic type in that it was present throughout the range of the host in Europe and generally at low levels. This concurred with the field observations of Scott (pers. comm. 1995a). Occasionally, severe rust symptoms were seen on patches of *H. pilosella.*

Field observations (Chapter IV) showed the uredinial (asexual) stage was predominant, with the main peak in field infection in spring to early summer followed by a smaller peak in autumn. A reduction in presence of rust sori over winter and over summer indicated the negative effects of cold and dry conditions respectively, on infection and sori survival.

Hieracium rust can overwinter in relatively cold Scottish highland conditions. During Edinburgh winters, urediniospore production could occur in periods of mild weather (Section IV.1). In most rust infected Scottish patches, spore production was noted in early spring (March). The presence of the sexual phase was not commonly observed. Survival of rust over winter was observed to occur in leaf tissue of *H. pilosella* (Section IV.1).

Survival of rust was also noted during a hot dry Mediterranean summer (Section II.1). European field surveys (Sections II.1 and IV.2) found that rust sori presence was sustained in summer where macro- or microclimatic conditions were sufficiently moist. The presence of tall grass next to some *H. pilosella* patches in Edinburgh was conducive to maintaining mid-summer levels of actively sporulating sori (Section IV.4).

Extrapolation would indicate that mid-summer activity of the rust would be greater in areas of higher rainfall and in microclimates sheltered by vegetation, for example tussock. Most activity of the rust would occur in spring and autumn. The presence of other varieties of *P. hieracii* on Lactuceae hosts in the Mackenzie country basin (Section I.4.2.b), demonstrates the ability of the rust species to reproduce and survive in low to moderate rainfall zones of the New Zealand high country.

Glasshouse experimentation showed that rust had a measurable negative effect on above and below ground growth of *H. pilosella*. Seven weeks after inoculation, infected plants had 20 to 40% less biomass than uninfected comparisons (Section III.9.c). Evidence in the literature (Section I.4.7) suggests that rust disease often exacerbates the effect of environmental stresses such as drought and cold. Both these stresses are prevalent in much of the *Hieracium* infested areas of the New Zealand high country. It is predicted that the effect of *Hieracium* rust infection on the host would be increased by these environmental stresses.

Impact on non Target Species

Host specificity testing showed no evidence of successful rust infection on species outside the subgenus *Pilosella* (Section III.8). This confirmed previous tests (Section I.5.1.b) and expectations from the literature (Section I.4.2.b).

Economic and Environmental Impact of Biological Control of *Hieracium*

Hieracium control would have great benefits for both the economic activities and the environment (Sections I.1.2.a and I.1.2.b respectively). To further investigate the possible environmental impact of a high degree of *Hieracium* control, the mineralisation of *Hieracium* plant parts was examined (Appendix 5). Indications were that there would not be a problem of immobilisation of nitrogen following the death of *Hieracium* patches and that the resulting decomposition of *Hieracium* may in fact provide establishing plants with mineral nutrients.

It is difficult to predict the effect of introduced rust on New Zealand *Hieracium* populations by extrapolation of glasshouse studies and field observations in Europe. The extent to which rust biological control would reduce *Hieracium* populations in New Zealand will not be known until after introduction. Research indicates that a short term dramatic effect is unlikely. Rust disease was not observed to cause the death of rosettes in field or glasshouse observations (Chapters III and IV). The effect was a reduction in host vigour, which will hopefully be sufficient to allow other plant species more opportunity to establish and survive in *Hieracium* infested areas.

Protocols

Variability in the pathogenicity of isolates was evident, therefore a combination of ten or so most pathogenic rust isolates should be chosen for introduction as biological control agents. Field inoculations or introductions of infected plants to New Zealand should be timed carefully according to season, spring time would be preferable for rust epidemiology.

The spread of rust throughout New Zealand high country could be facilitated by human intervention. Rust infected plants can survive in postage (Section II.2.2).

Urediniospores were able to maintain viability after two to three weeks storage at room temperature (pers. obs.) and urediniospores remain viable under liquid nitrogen storage without cryoprotectant (Scott, pers. comm. 1995a). Therefore urediniospores could be produced year round and stored until favourable conditions for field dissemination.

Epilogue

In April 1995, rust was found on *H. pilosella* grown at Lincoln, New Zealand (S. Helfer, J.E. Jenkins, T.A. Jenkins and D. Scott, unpublished observation). This isolate of *P. hieracii* var. *piloselloidarum* has since been included in experiments to supplement knowledge gained from this project and to assess the potential of the New Zealand strain for controlling *Hieracium* in New Zealand. Some of the identified deficiencies in the project that are now being corrected are measuring the effect of the rust on *H. pilosella* growth in the field and assessing the level of genetic resistance to the rust isolate that might exist in New Zealand populations of *H. pilosella*. Experiments leading to technology for efficient dissemination of the rust throughout the New Zealand high country are also being investigated. Several rust sori have also been noted on *H. praealtum* growing in the field near rust-infected *H. pilosella* at Lincoln (unpublished observation, October 1995).

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Appendix 1 : Logistic regression statistics for Section V.3.1

Table A.1. (from Section V.3.1) Logistic Regression on number of leaves ($\log_{10}(x + 1^{-10})$ transformed) infected by isolates on detached leaves.

Variable	df	Estimate	Standard Error	CHI^2	p > CHI
INTERCEPT	1	-2.01	1.14	3.09	0.079
ISOLATE	69			122	0.0001
DAY	47			57.6	0.1375

Table A.2. (from Section V.3.1) Logistic Regression on number of sori produced by isolates on detached leaves.

Variable	df	Estimate	Standard Error	CHI^2	p > CHI
INTERCEPT	1	20.3	20300	10^{-6}	0.9992
ISOLATE	69			65	0.6011
DAY	47			48	0.4368

Appendix 2 : Full data of Section V.3.4

Table A.3. (from Section V.3.4) Results of the inoculation of British rust isolates onto one rosette of each of *H. pilosella* localities HpilLRd and HpilHOs. Only the 135 isolates successfully infecting the hosts are listed.

Ranking	Isolate Code	No. of Sori on host HpilLRd	No. of Sori on host HpilHOs	Hosts Infected	Average No. of Sori	Ranking in 1992[a]
1	BRIT12	58	38	h,l	48	1=
2	BRIT21	47	48	h,l	47.5	3
3=	BRIT330	78	12	h,l	45	
3=	BRIT627	31	59	h,l	45	
5	BRIT341	89	0	l	44.5	
6	BRIT1120	87	0	l	43.5	
7	BRIT429	39	45	h,l	42	
8=	BRIT170	44	38	h,l	41	
8=	BRIT278	40	42	h,l	41	
8=	BRIT397	79	3	h,l	41	
11	BRIT459	73	8	h,l	40.5	
12=	BRIT348	80	0	l	40	
12=	BRIT577	80	0	l	40	
14	BRIT757	48	28	h,l	38	
15	BRIT389	75	0	l	37.5	
16	BRIT1100	74	0	l	37	
17	BRIT491	36	36	h,l	36	
18	BRIT599	26	44	h,l	35	
19	BRIT259	69	0	l	34.5	
20=	BRIT495	65	0	l	32.5	

Table A.3. (cont.)

Ranking	Isolate Code	No. of Sori on host HpilLRd	No. of Sori on host HpilHOs	Hosts Infected	Average No. of Sori	Ranking in 1992[a]
20=	BRIT565	49	16	h,l	32.5	
20=	BRIT910	35	30	h,l	32.5	
20=	BRIT1087	53	12	h,l	32.5	
24	BRIT1122	50	14	h,l	32	
25	BRIT387	0	62	h	31	
26	BRIT866	0	60	h	30	
27=	BRIT191	0	59	h	29.5	
27=	BRIT609	59	0	l	29.5	
29	BRIT230	58	0	l	29	
30=	BRIT117	0	57	h	28.5	
30=	BRIT315	57	0	l	28.5	
32=	BRIT260	35	21	h,l	28	
32=	BRIT544	56	0	l	28	
34	BRIT886	0	55	h	27.5	
35	BRIT175	54	0	l	27	
36=	BRIT99	53	0	l	26.5	
36=	BRIT361	40	13	h,l	26.5	
38=	BRIT207	52	0	l	26	
38=	BRIT274	0	52	h	26	
40=	BRIT147	3	48	h,l	25.5	
40=	BRIT975	0	51	h	25.5	
42=	BRIT218	50	0	l	25	
42=	BRIT873	0	50	h	25	
42=	BRIT64	0	50	h	25	10
45=	BRIT103	49	0	l	24.5	
45=	BRIT574	49	0	l	24.5	
45=	BRIT53	49	0	l	24.5	1=
48=	BRIT399	0	48	h	24	
48=	BRIT504	17	31	h,l	24	
50=	BRIT449	13	33	h,l	23	
50=	BRIT466	0	46	h	23	
52	BRIT630	45	0	l	22.5	
53=	BRIT288	0	44	h	22	
53=	BRIT865	44	0	l	22	
53=	BRIT991	0	44	h	22	
56=	BRIT711	18	25	h,l	21.5	
56=	BRIT883	43	0	l	21.5	
58=	BRIT275	11	31	h,l	21	
58=	BRIT26	42	0	l	21	4
60	BRIT60	0	41	h	20.5	7
61=	BRIT896	40	0	l	20	
61=	BRIT1058	0	40	h	20	
63=	BRIT120	0	39	h	19.5	
63=	BRIT148	39	0	l	19.5	
63=	BRIT239	21	18	h,l	19.5	
66=	BRIT137	0	37	h	18.5	
66=	BRIT367	37	0	l	18.5	
68=	BRIT166	35	0	l	17.5	
68=	BRIT610	35	0	l	17.5	
70=	BRIT453	0	34	h	17	
70=	BRIT1140	34	0	l	17	

Table A.3. (cont.)

Ranking	Isolate Code	No. of Sori on host HpilLRd	No. of Sori on host HpilHOs	Hosts Infected	Average No. of Sori	Ranking in 1992[a]
70=	BRIT62	34	0	l	17	9
73	BRIT462	33	0	l	16.5	
74=	BRIT351	0	31	h	15.5	
74=	BRIT417	16	15	h,l	15.5	
76	BRIT496	0	30	h	15	
77=	BRIT78	29	0	l	14.5	
77=	BRIT209	0	29	h	14.5	
77=	BRIT210	29	0	l	14.5	
77=	BRIT269	0	29	h	14.5	
77=	BRIT328	29	0	l	14.5	
77=	BRIT358	0	29	h	14.5	
77=	BRIT522[b]	29	0	l	14.5	
84=	BRIT98	28	0	l	14	
84=	BRIT887	0	28	h	14	
86=	BRIT169	0	27	h	13.5	
86=	BRIT385	0	27	h	13.5	
86=	BRIT528	27	0	l	13.5	
86=	BRIT1041	0	27	h	13.5	
90	BRIT32	0	26	h	13	5
91	BRIT365	0	25	h	12.5	
92=	BRIT223	6	18	h,l	12	
92=	BRIT624	24	0	l	12	
94=	BRIT138	23	0	l	11.5	
94=	BRIT502	0	23	h	11.5	
94=	BRIT551	0	23	h	11.5	
97=	BRIT176	22	0	l	11	
97=	BRIT868	0	22	h	11	
99	BRIT512	21	0	l	10.5	
100=	BRIT121	20	0	l	10	
100=	BRIT134	20	0	l	10	
102	BRIT382	19	0	l	9.5	
103=	BRIT220	17	0	l	8.5	
103=	BRIT346	17	0	l	8.5	
103=	BRIT556	17	0	l	8.5	
103=	BRIT648	17	0	l	8.5	
107	BRIT507	0	16	h	8	
108	BRIT874	15	0	l	7.5	
109=	BRIT271	0	14	h	7	
109=	BRIT320[b]	0	14	h	7	
109=	BRIT396	0	14	h	7	
109=	BRIT467	14	0	l	7	
109=	BRIT923	14	0	l	7	
109=	BRIT1114	10	4	h,l	7	
115=	BRIT228	13	0	l	6.5	
115=	BRIT369	13	0	l	6.5	
115=	BRIT616	0	13	h	6.5	
115=	BRIT925	0	13	h	6.5	
119=	BRIT352	0	11	h	5.5	
119=	BRIT1127	11	0	l	5.5	
121	BRIT306	3	7	h,l	5	
122	BRIT73	0	9	h	4.5	

Table A.3. (cont.)

Ranking	Isolate Code	No. of Sori on host HpilLRd	No. of Sori on host HpilHOs	Hosts Infected	Average No. of Sori	Ranking in 1992[a]
123=	BRIT316	0	8	h	4	
123=	BRIT520[b]	0	8	h	4	
123=	BRIT864	8	0	l	4	
126=	BRIT412	0	7	h	3.5	
126=	BRIT57	7	0	l	3.5	6
128	BRIT620	6	0	l	3	
129	BRIT182	0	5	h	2.5	
130=	BRIT1099	3	0	l	1.5	
130=	BRIT52	0	3	h	1.5	8
132=	BRIT325	0	2	h	1	
132=	BRIT596	0	2	h	1	
134=	BRIT241	0	1	h	0.5	
134=	BRIT731	0	1	h	0.5	

[a] Applicable to the ten 1992 collections.
[b] These isolates were collected from *H. flagellarum* hosts in the field.

Appendix 3 : Full results of Section V.3.7

Table A.4. (From Section V.3.7) Full results of inoculations of five Irish and two British isolates onto a range of New Zealand *H. pilosella* populations.

		ISOLATE						
Locality		ÉIRE14	Éire 29	Éire 32	Éire 45	Éire 66	IRi	AlcIII
HpilLRd	detached lvs	21,9,4,3	10,5,6,1	1,2,9,3	2,7,2,6	0,5,3,4	0,2,5,9	0,0,6,5
	whole rosettes	131,143	87,155	187,54	26,16	29,38	0,47	4,35
HpilGPs	detached lvs	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0	0,0,0,0
	whole rosettes	0,0	0,0	0,0	0,0	0,0	0,0	0,0
HpilMJt[a]	detached lvs	12,13,20,9	35,44,5,3	8,6,5,9	2,18,16,6	11,5,1,1	3,4,0,12	16,3,0,6
	whole rosettes	116,0	0,74	150,0	90,73	83,0	0,15	0,8
HpilHOs[a]	detached lvs	47,9,11,12	19,22,3,3	37,15,9,10	7,24,13,12	7,5,12,20	2,0,12,3	1,0,8,0
	whole rosettes	33,93	50,21	16,52	48,83	0,65	19,38	61,0
HpilLRu[a]	detached lvs	4,15,49,11	23,17,14,5	9,6,12,13	25,37,6,5	10,0,16,21	0,3,6,7	0,4,0,6
	whole rosettes	13,104	41,86	56,43	0,40	63,17	26,13	24,0

[a] Rosettes from these localities correspond to variants identified by isozyme electrophoresis; see text for details.

Appendix 4 : Ploidy levels of New Zealand *Hieracium*

Table A.5. (from Section VII.1.3) Ploidy levels of New Zealand *H. pilosella* collections.

TAXA[a]	LOCALITY CODE	PLOIDY LEVEL
H. pilosella (m)	HpilDRd	5X
H. pilosella (m)	HpilTUa	5X
H. pilosella (m)	HpilLVa	5X
H. pilosella (m)	HpilHSp	5X
H. pilosella (m)	HpilJPa	5X
H. pilosella (m)	HpilHBp	5X
H. pilosella (t)	HpilCRa1	5X
H. pilosella (m)	HpilCRa2	5X
H. pilosella (m)	HpilLLy	5X
H. pilosella (m)	HpilLCo	5X
H. pilosella (m)	HpilLRd	5X
H. pilosella (m,p,t)	HpilLCl	5X
H. pilosella (m)	HpilHEr	5X
H. pilosella (m)	HpilGPs	5X
H. pilosella (m)	HpilMJb	5X
H. pilosella (m)	HpilMJt	5X
H. pilosella (m)	HpilMJw	5X
H. pilosella (m)	HpilLTe	5X
H. pilosella (m)	HpilHOs	5X
H. pilosella	HpilDKc	5X
H. pilosella (m)	HpilTWo	5X
H. pilosella (m)	HpilGEr	5X
H. pilosella (m)	HpilMAs2	5X
H. pilosella (m)	HpilMAs1	5X
H. pilosella (m)	HpilLRu	5X
H. pilosella (m)	HpilTWz	5X
H. pilosella (m)	HpilCAv	5X
H. pilosella	HpilODs	5X
H. pilosella (m)	HpilHAs	5X,6X
H. pilosella (m)	HpilSCs2	5X
H. pilosella (m)	HpilCAn	5X
H. pilosella (m)	HpilCVa	5X
H. pilosella (m)	HpilSCs1	5X
H. pilosella	HpilTHs	5X

[a] Letters in brackets after binomials indicate that specimens matched subspecies described in the Flora Europaea by Sell and West (1976).

(m)=subsp. *micradenium*

(p)=subsp. *pilosella*

(t)=subsp. *trichosoma*.

Table A.6. (from Section VII.1.3) Ploidy levels of New Zealand *Hieracium* spp. collections.

TAXA[a]	LOCALITY CODE	PLOIDY LEVEL
H. aurantiacum (c)	HaurPPa	4X
H. caespitosum (c)	HcaeLCl	4X,5X
H. caespitosum (c)	HcaeMJt	5X
H. caespitosum (c)	HcaeDKc	-
H. caespitosum (c)	HcaeMJw	-
H. caespitosum (c)	HcaeLRd	-
H. caespitosum (c)	HcaeLTe	-
H. caespitosum (c)	HcaeMAs	-
H. lepidulum	HlepCRa	3X
H. praealtum (t)	HpraCRa	4X
H. praealtum (p)	HpraLCl	5X
H. praealtum (t)	HpraMJt	5X
H. praealtum (t)	HpraMJs	5X
H. praealtum (t)	HpraTWo	4X
H. praealtum (t)	HpraLRu	4X
H. praealtum (t)	-	-
H. praealtum (t)	-	-
H. praealtum (t)	-	-
H. praealtum (t)	-	-
H. praealtum	-	-
H. praealtum	-	-
H. praealtum	-	-
H. praealtum (t)	-	-
H. praealtum (t)	-	-
H. x *stoloniflorum*	HstoCRa	6X
H. x *stoloniflorum*	HstoMJs	6X
H. x *stoloniflorum*	HstoLTe	6X

[a] Letters in brackets after binomials indicate that specimens matched subspecies described in the Flora Europaea by Sell and West (1976).

For *H. aurantiacum* : (c)=subsp. *carpathicola.*
H. caespitosum : (c)=subsp. *caespitosum.*
H. praealtum : (p)=subsp. *praealtum.*
H. praealtum : (t)=subsp. *thaumasium.*

Appendix 5 : Nitrogen mobilisation from decomposing *Hieracium* plant matter

INTRODUCTION

The mineralisation of nitrogen from decomposing leaves, stolons and roots of *H. pilosella* and *H. praealtum* was investigated under laboratory conditions. The aim was to assess the relative rate of mineralisation to provide information on the likely release or immobilisation of mineral nutrients following the death of *Hieracium* such as may occur with successful biological control.

MATERIALS AND METHODS

Green leaves, senescing leaves, underground parts and stolons were harvested from field grown *H. pilosella*, *H. praealtum* and for comparison, *T. officinale*. All plants had been transplanted from the Mackenzie country and grown for one year at Lincoln, in Templeton silt loam without fertiliser application. Senescing leaves were selected on the basis of no visible green tissue being present, and that they were not contaminated with soil. Other plant parts were cleaned to remove soil, then washed lightly with distilled water. For *H. pilosella* and *H. praealtum*, underground parts were comprised of roots and rhizome, and harvested stolons had their leaves removed. *T. officinale* lacks stolons and a rhizome, and the root system consists mainly of a large taproot.

Samples were completely air dried for 48 h at 60°C and mill ground to a fine powder. Nitrogen analysis followed the methods of Greenfield (1993). The total nitrogen content of each sample was determined by Kjehldahl digestion, Hg catalyst, salt-acid ratio 0.7 and 3 h digestion period (Bremner, 1965). Amounts of each sample were placed in small conical flasks (50ml) such that 5 mg of total nitrogen was included in each flask. Twelve replicates of each species plant part were set up. Plant matter in each flask was dry mixed with 10 g of sterile coarse sand; then moisture levels were adjusted to 20% by weight with sterile double distilled (SDD) water. Flasks were sealed with thin polythene film, then kept in darkness in a 20°C incubator. Moisture level was kept at 20% throughout the duration of the experiment by occasional

addition of SDD water. Three replicate flasks of each species plant part were removed at 0, 4, 8 and 12 weeks after commencement; each flask received 46 ml of 2N KCl and was left to stand for 24h with occasional shaking; mineral nitrogen content was calculated using the MgO, Devarda's alloy and steam distillation method (Bremner, 1965).

RESULTS AND DISCUSSION

Leaves, stolons and roots of *H. pilosella* all demonstrated mobilisation of nitrogen when set up for decomposition in flasks. The leaves and roots of *H. praealtum* also demonstrated mobilisation of nitrogen though with this species stolon decomposition apparently immobilised nitrogen. Leaves and stolons of *Taraxacum officinale* were included in the experiment for some comparison and apparently mobilised nitrogen faster than the two *Hieracium* species.

Table A.7 Mineralisation of nitrogen from plant parts of *H. pilosella*, *H. praealtum* and *Taraxacum officinale*.

Species	Plant Part	Total N (%)	Initial Min N (%)	Min N(%) 4 Weeks	Min N(%) 8 Weeks	Min N(%) 12 Weeks
H. pilosella	leaves	3.1	2.3	20	26	18
	senescing lvs.	2.9	3.9	34	34	22
	roots	1.5	3.5	8	13	8
	stolons	2.9	3.5	27	37	10
H. praealtum	leaves	3.7	2.2	22	34	27
	senescing lvs.	2.5	4.7	13	20	23
	roots	2.0	3.5	11	22	21
	stolons	1.9	6.2	3	2	6
T. officinale	leaves	3.4	3.4	55	64	49
	senescing lvs.	2.0	6.8	4	12	9
	roots	1.1	5.2	3	11	9

There was no indication that immobilisation of nitrogen would follow the killing of *H. pilosella* or *H. praealtum* plants. It is expected that sudden killing of plants would not result in instant availability of tissue for decomposition. When *Hieracium* subgenus *Pilosella* leaves begin senescing they typically remain attached to the rosette. Even in relatively moist glasshouse conditions the brown senescing leaves can be attached for around two weeks before incorporation into the soil. The mineral composition of

such senescing leaves is remarkably high. Dry weight nitrogen percentage of senescing *H. pilosella* leaves was 91% of that in live leaves; for *H. praealtum* the value was 69% and with *T. officinale* just 41%. This indicates that senescence is a gradual process, perhaps occurring relatively slowly in the two *Hieracium* species. The senescing leaves were also found to mobilise nitrogen in decomposition experiments in flasks.

Leaf senescence was defined by Finney (1979) as a number of irreversible processes that are involved in leaf death. In a biochemical study of leaf senescence, Finney (loc. cit.) measured loss of chlorophyll, reduction of protein-nitrogen content and an increase in α-amino-nitrogen. The senescing leaves used in this experiment were completely brown though not definitely dead. The total nitrogen content is reasonably high in the senescing leaves indicating that senescence may not have been complete.

It is important to note that the decomposition and mineralisation of rust infected leaves is likely to differ from that of uninfected leaves. McKenzie and Hudson (1976) found that rusted leaves of poplar, plum and wheat generally had more fungi present on the phylloplane than non-rusted leaves. As mentioned in Section III.9.1.d rust infection can cause the early senescence of leaves, including through the entry of secondary pathogens (see Fig 3.16). The composition of infected tissue would also be affected with a proportion of fungal tissue.

Decomposition of tall tussock grass (*Chionochloa* spp.) litter in the New Zealand high country, is quite slow with 95% decay of *Chionochloa rigida* dead leaf compartments only occurring after several years (O'Connor, 1983). O'Connor noted that leaf litter orientation and leaf structure had significant effects on their decomposition rate. Above ground plant parts of *Hieracium* spp. would probably decompose faster than *Chionochloa* tussock tillers as they are richer in nitrogen and have greater contact with the soil surface. O'Connor (1983) stated that the differences in *Chionochloa* decomposition rate due to site factors were substantial. Site factors such as moisture, temperature and fertility would also influence *Hieracium* decomposition.